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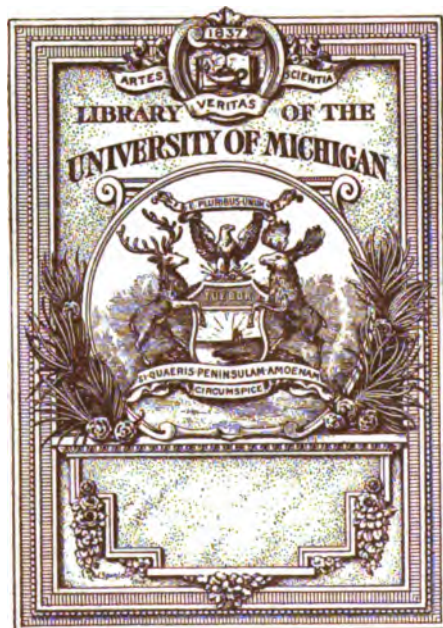
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A STUDY OF THE SPORANGIA AND GAMETOPHYTES OF SELAGINELLA APUS AND SELAGINELLA RUPESTRIS

A DISSERTATION

SUBMITTED TO THE FACULTIES OF THE GRADUATE SCHOOLS OF ARTS,
LITERATURE, AND SCIENCE, IN CANDIDACY FOR THE
DEGREE OF DOCTOR OF PHILOSOPHY

(DEPARTMENT OF BOTANY)

BY

FLORENCE MAY LYON

CHICAGO

1901

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A STUDY OF THE SPORANGIA AND GAMETOPHYTES
OF SELAGINELLA APUS AND SELAGINELLA
RUPESTRIS.

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY.
XXXI.

FLORENCE MAY LYON.

(WITH PLATES V-IX)

SELAGINELLA APUS.

SPORANGIUM.—It is by no means an easy matter to determine the origin of the sporangium in *S. apus*. It is quite possible, from a number of slides, to select a series which shall seem to prove that the archesporium consists of two superficial cells originating just above the axil of a sporophyll (*figs. 3, 4*). It is equally easy to procure evidence that a single epidermal cell initiates the sporangium (*figs. 1, 2, 5*). This single cell may be either upon the sporophyll or removed by several intervening cells from its base. The exact line of demarcation between sporophyll and axis is indeterminate. The difficulty lies in the fact that until the sporangium is well established, consisting of some half dozen or more cells, there are almost no indications in the structure, or size, or staining qualities of these cells to distinguish them from the vegetative tissue. Moreover, a sporophyll originates in close proximity to a sporangium (*fig. 5*), and at about the same time and until it has established an apical cell there is no way of distinguishing it from a young sporangium. These facts render cross and tangential sections well nigh useless for an interpretation of the earliest stages. From serial radial, *i. e.*, vertical, sections of the strobilus, if cut with due reference to the phyllotaxy, it is possible to form conclusions by comparison of the series of sporangia and sporophylls in the same rank and of different ages that appear in an exact median section. It is evident that the number of such

sections which may be obtained by ordinary means is very limited. I found it necessary to imbed the strobili singly, examine each section as its plane approached the median region, and constantly alter the angle of the paraffin block in the microtome, until by the vascular strand of the axis and the leaf traces of the older sporophylls I could estimate approximately the section sought. Even with these precautions, I was not infrequently doubtful as to whether a cell that appeared in the right place theoretically for an archesporium might not be the initial cell of a sporophyll from the next rank that a slightly oblique section would display. These explanations are necessary, for after much painstaking study of many sections of tips of very young strobili, I find myself becoming less certain of there being a definite rule governing the initial phases of the sporangial growth. In many median longitudinal sections cut in the manner described above, a single epidermal cell projects from the surface in a vertical line between the youngest sporophyll and the apical cell of the strobilus. Occasionally it takes a deeper stain than the neighboring cells. There are usually from ten to twelve cells between it and the apical cell, and two or three between it and the subtending sporophyll (*fig. 5*). Quite as often this projecting papilla is composed of *two* cells of equal size lying in the vertical line (*figs. 3, 4*). In either case periclinal walls are formed, cutting off one or two cap cells (*figs. 2, 5, 6*). From the hypodermal cell or cells, thus formed, originates the sporogenous tissue. From the cap cell, which divides much more rapidly, the sporangium wall is formed. It is possible, I think, to determine in sporangia of various stages, nearly up to maturity, whether in a given case it originated from a single epidermal cell or from the two superimposed cells. In the second case, the complex of cells resulting from each of the two primary cells consists of more regular radial rows, and there is a quite definite plane of cleavage between the two groups. The only other interpretation of appearances like *figs. 6* and *8* is that the primary single archesporial cell divided first anticlinally, thus producing the superimposed cells, each of which then cuts

off a cap cell. I have sought in vain, however, to establish that the first two are sister cells. When the sporangium consists of from four to six cells (*figs. 6, 7, 8*), the hypodermal cells assume a different appearance when stained, which distinction is maintained thenceforth in the development, and establish their identity as primary sporogenous cells. There is no apparent regularity in the order or plane of their division, with the exception above stated. The tapetal cells are differentiated very early in the history, and are the peripheral sporogenous cells which assume a more symmetrical shape and regular arrangement (*fig. 10*). A few of the cells in this layer, lying directly above the pedicel of the sporangium, appear to be derived from the vegetative cells in that region, and are not the lineal descendants of the archesporial cell.

Simultaneously with the differentiation of the tapetum the sporangium wall divides into two layers by anticlinal partitions (*fig. 10*). The outer layer soon surpasses the inner in the size of its cells (*fig. 11*). A pedicel develops by the multiplication of the sterile cells next to the axis and beneath the sporogenous mass. The megasporangium cannot be distinguished from the microsporangium until the moment when the cells of the sporogenous tissue cease to divide, separate from one another, and float in the sporangium. At this period, if one or two cells are more regular in form, stain more deeply, and possess a larger nucleolus, they are megaspore mother cells (*figs. 13, 35, 36*). The microspore mother cells are not distinguishable until the karyokinetic figures develop that precede their division into spores. A comparative count of the microspore mother cells in this stage and the cells in a corresponding megasporangium indicates that there is at least one more period of cell division in the former than in the latter. A cytological study may determine other means of identifying the two.

MEGASPORES.—The megaspore mother cell becomes nearly isodiametric, enlarges, stains deeply, and lies near the tapetum (*figs. 13, 35*). Apparently the mother cell never originates far within the sporogenous tissue but always near its periphery.

Infrequently two mother cells occur, and these may go through all the later stages of development, thus forming eight spores in a megasporangium. Normally the membrane of the single mother cell becomes distended by the imbibition of fluid that has been poured into the sporangial cavity from the tapetum. Two successive divisions follow each other with great rapidity. The spindle of the first division I have seen but a few times; the two that follow, I saw more frequently (*figs. 16, 17*). These are extremely small and delicate, occupy the middle of the cell, and are soon much obscured by fibers which arise in the surrounding protoplasm. These fibers at first radiate in all directions from no definite center, but later assume the form of a sextuple spindle with the four daughter nuclei at the poles. No cytological work was attempted, beyond determining the method by which the spores originate, but it is evident even from a cursory examination that there is some connection between the enveloping spindle fibers and the two lumps lying against the nucleus which are represented in *figs. 11, 13, 15*. In the meantime there has appeared a new membrane just within the mother cell wall (*fig. 15*). It is very delicate, but of unequal thickness, and can be detected only in particularly fortunate sections. Whether this membrane arises by cleavage from the mother cell wall, or *de novo* from the protoplasm of the cell, is necessarily a matter of speculation in an object so small. Nuclear plates form across the spindles, and the mother cell divides into four spores tetrahedrally arranged. At the moment of their separation the nuclei lie near the bases of the spores, which correspond in position to the poles of the sextuple spindle, but soon move toward the apices (*figs. 16-18, 22*). As the spores increase in volume, by their pressure outward, and by a folding inward on the part of the irregularly thickened special mother cell membrane above described, the tetrad presents the appearance of a four-lobed body surrounded by a single envelope whose continuity is not broken, floating in the fluid that fills the distended spore mother cell membrane (*figs. 17-19*). Either the special mother cell membrane itself thickens, or a new coat is

formed upon its inner surface. This is the exospore (*figs. 20-22*). The spores increase in volume, but the exospore expands so much more rapidly than does the protoplasmic content, that the latter is left in the apical portion of the spore cavity as a tenuous spherical vesicle of protoplasm filled with a limpid fluid (*fig. 37*). A single very small nucleus, in which with difficulty may be seen one or two nucleoli, lies in the region nearest the spore apex. The rest of the spore cavity between the vesicle and the exospore is filled with a limpid fluid of the same nature as that which occupies the space between the tetrad and the mother cell membrane. Soon hair-like radiations appear traversing these fluid regions (*figs. 42, 43*), whose general direction is from the spore mother cell membrane toward the center of the tetrad. A thick layer which stains very deeply appears upon the inner face of the exospore (*fig. 37*). The latter maintains its more rapid rate of growth and soon afterward is widely separated from this layer by a space filled with the liquid and the radial fibrillae (*figs. 38, 42*). The sculpturing of the exospore begins almost immediately upon its inception. The spines are laid down by depositions of matter derived from the liquid between the exospore and the mother cell membrane (*figs. 42-46*).

As the megaspores increase in size, they are forced apart by the liquid in which they float seeping in between them (*figs. 38, 42, 43*) and the exospore is ruptured between the spores in such manner that each presents the appearance of a tetrahedron with a hemispherical base and three plane triangular faces. The tearing apart of the spores leaves a trefoil-shaped cleft extending from the apex along the three ridges between the triangular faces, and bounded by the flaring flaps of the torn exospore (*fig. 58*).

The sterile mother cells in part disappear by dissolving in the slimy fluid in the sporangium cavity, but not until the exospore is well developed. Some persist however throughout the development of the gametophyte and perhaps may grow slightly; they never divide.

FEMALE GAMETOPHYTE.—The spore is but a small fraction of

its final volume when the sexual generation begins, thus overlapping the asexual (*fig. 44*). The initial steps of the female gametophyte development are the rapid expansion, without corresponding increase in thickness, of the protoplasmic vesicle, and the division of its nucleus. The nuclei divide by karyokinesis, and with each successive division become larger (*figs. 45, 46*). The thick envelope surrounding the vesicle stretches, becoming proportionately thin as its surface increases, until it comes to lie against the inner surface of the exospore. At this stage it consists of two distinct layers, the endospore and mesospore, both mere films which may be readily detected, as they stain differently and are easily torn apart (*fig. 46*). The gametophyte at this stage thus consists of (1) exospore, still growing; (2) mesospore; (3) endospore; (4) the protoplasmic vesicle, consisting of a very thin and homogeneous layer of protoplasm applied to the inner surface of the endospore, in whose apical region are imbedded numerous large, ovate, flattened nuclei; and (5) a large central vacuole filled with a watery fluid in which are suspended many oil drops.

Beyond the increase in the number of nuclei, which preserve about the same relative distance from one another, there is no further change until the spore membranes complete their growth. When the maximum size is reached, fibrillae arise in the protoplasmic vesicle at the apex, and radiate downward over its surface, blocking it off into irregular areas, each of which contains one or more nuclei (*figs. 49, 51, 52*). Simultaneously the protoplasm becomes invaded with masses of granular matter, and encroaches on the space occupied by the vacuole. Nuclear divisions take place radially, and so rapid is this process that frequently the spindle fibers of three and four generations of nuclei may be seen in a single section. The fibrillar radiations permeate the protoplasm, keeping pace with its increase in thickness. As the central vacuole diminishes in size, its contents change in appearance from an emulsion to a turbid fluid thickened with granules (*fig. 53*). The protoplasmic fibrillae apparently are concerned with the distribution

of this nutriment before cell walls are formed. They have the appearance of streams bearing granules. The final and penultimate divisions of the nuclei are distinguished by the appearance of nuclear plates and spindles of quite different appearance, which result in the formation of definite cells with cell walls, each containing a single nucleus (*figs. 26-28*). At this stage, each nucleus is so surrounded by a mass of proteid as to be completely obscured. The only cells not thus gorged are a limited number which lie in the upper layer nearest the apex, beneath the trefoil-shaped cleft in the exospore (*fig. 54*). By continual multiplication they form a prothallial cushion, which widens the breach and bulges through. These cells are much smaller than those filled with the food supply. There may be from three to five layers in this cushion. There is no diaphragm between the region designated the prothallial cushion and the mass of storage cells underlying it.

ARCHEGONIA.—A few cells in the prothallial cushion soon become conspicuous by reason of their large nuclei. Each divides by two periclinal walls, forming a tier of three cells. The uppermost of these divides by two anticlinal walls at right angles to each other into four cells. These again, by periclinals, form the four cover and the four neck cells. The middle one of the original tier does not divide and becomes directly the single neck canal cell. The lowermost divides into the egg and the ventral canal cell (*figs. 29-32*). A suggestive irregularity sometimes occurs in the last mentioned division. The central cell, that is, the lowermost cell of the original tier, may divide in such fashion that the egg and its sister, the ventral canal cell, may lie side by side in the venter of the archegonium, instead of in the normal fashion of ventral canal cell above the egg (*fig. 32*). The cover cells project very little from the surface of the prothallium. Thus the archegonia are imbedded in the surrounding tissue, whose cells in immediate contact with the egg and the ventral canal cell become more or less modified in form. The neck canal cell pushes up like a wedge, spreads apart the four neck cells, and dissolves. The ventral canal cell

also disappears (with a possible exception noted hereafter), and the egg lies free in the venter. There is a large receptive spot on the oosphere, and its nucleus is not centrally placed (*fig. 33*). I have never seen more than five archegonia in a single gametophyte.

FERTILIZATION.—Not only are the archegonia formed in the unshed spores, but frequently, at least, fertilization and the early phases of the sporophyte development take place while the sporangium with its prothallus are still in the strobilus. The strobili cease to grow, fade, and may separate from the plant before fertilization, but the spores do not fall from the sporangium. It was not until I had collected from the soil several hundred spores which had been shed, with the expectation of finding fertilization stages, that I thought to examine the withered strobili. Almost without exception in these I found embryos, whereas *in no case* have I found any evidence of fertilization in the spores that are shed.

FURTHER DEVELOPMENT OF THE MEGASPORANGIUM.—The glandular tapetum is very active until the megaspores have stored their maximum amount of nutriment for the growth of the embryo. At this stage they quite fill the sporangium cavity, which in consequence has assumed a four-lobed appearance. The tapetum then declines in importance. Its cells collapse and form a pavement-like layer. The outer layer of wall cells becomes greatly modified. In section it appears precisely like a similar section of the annulus of a leptosporangiate fern (*fig. 52*). Four areas of the larger thick-walled cells, corresponding to the protuberances caused by the spores lying within, are separated from one another by narrow strips of small cells with thin walls. The latter are the lines of dehiscence. The sporangium splits into two valves along these lines, but the halves do not separate so widely as to allow fertile spores to escape. Apparently they may open and close more than once. The sporangium appears fresh and active, and its wall contains chlorophyll until after fertilization has occurred. With the decline of the tapetum the lower stratum of the wall becomes

more vigorous, as does a group of cells that lies just above the pedicel, and which projects into the sporangial cavity (*fig. 58*). This cushion is in close relation to the vascular strand and probably facilitates the supply of nutriment to the sporangium wall, until the embryos begin to form.

Frequently I have found microspores (in which the spermatozoids had formed) within the megasporangium at the period of fertilization, and it is possible that the microspores are hurled into a gaping megasporangium when ejected from the microsporangium.¹ This is rendered more probable by the frequent occurrence of microspores caught in the angles between sporophylls and stem. Moreover, if plants that have become somewhat dry be profusely watered, the mature microsporangia open explosively and discharge spores.

MICROSPORES.—The microspores of *S. apus* are much smaller than those of *S. Kraussiana* and *S. Martensii*. They early develop a pebbled, thick exospore, which causes much trouble in imbedding and sectioning (*fig. 69*). Moreover, a comparatively small number, in proportion to the immense output, mature. Curious aberrations in growth are constantly found. It was necessary to study mature gametophytes discharging spermatozoids and trace back the different stages to the mother cells. Frequent comparative measurements finally afforded a clew to detecting abnormalities in the early stages.

Not more than five sixths of the potential mother cells divide into spores; the others rapidly disappear. The division is accomplished in a manner analogous to the division of the megaspore mother cell, which is very little larger. Two spore coats develop, a thick spiny exospore, and the delicate membranaceous endospore. The microspores are shaped like the

¹I have examined *S. Martensii*, *S. Kraussiana*, and *S. denticulata*, growing in the greenhouse, with reference to this point. The two former do not shed their strobili, and I have found loose megaspores containing sporophytes in the soil on the benches where the plants are growing. The last mentioned species, on the other hand, sheds its strobili in profusion. I find, however, that their spores are invariably sterile and aborted, and therefore am unable to form an opinion as to whether this shedding is the normal habit, or due to cultivation in an unnatural environment.

megaspores, but, unlike the latter, separate from one another as soon as the exospore develops. As was the case with the female gametophyte, the sexual generation begins before the microspore has ceased growing. At the moment that the microspores separate from one another, each possesses a thick and a thin coat, a layer of protoplasm parietally placed, with one nucleus, and a central cavity filled with fluid (*fig. 68*). The nucleus increases in size and divides. This process often takes place in that part of the spore where one of the lateral ridges meets the hemispherical base (*fig. 78*). The protoplasm increases in quantity and encroaches on the central vacuole. Granules of various sizes make their appearance in the cavity and in the surrounding protoplasm. These bodies stain precisely like the nuclei and the more regular ones may be mistaken for them, as I frequently discovered in the early period of my own work (*fig. 77*). By precautions in decolorizing, the presence of nucleoli always distinguish the nuclei from the granular masses. These bodies are formed by the agglomeration of many smaller granules. One of the nuclei formed by the first division remains against the wall. It may grow larger and the protoplasm immediately surrounding it is somewhat denser, but no wall separates it from the rest of the spore. This may be the vegetative prothallial cell (*figs. 70, 75*). The other nucleus passes into the center of the spore. The protoplasm that envelops it sends out radiating processes that incompletely divide the spore cavity into irregular chambers, each of which is filled with granular masses of various sizes and shapes (*figs. 76, 80, 81, 83*). These strands of protoplasm are continuous with a thin layer in contact with the endospore. The central nucleus with its envelope of protoplasm divides into two cells which usually separate from each other (*figs. 79-83*). By repeated division of each of these cells a complex results which consists of two uniform masses of sperm mother cells. There is no law of sequence which the cells follow in dividing, although the final product consists of cells very regularly arranged (*figs. 95-97*). The male

gametophyte then is made up of one prothallial cell and a naked mass of sperm cells, which later come to float in the slime produced by the disorganization of the food granules. Sometimes at this stage the large deeply stained prothallial cell may be seen flattened against the endospore (*fig. 97*). There are about 128 sperm cells. The exospore splits along the three ridges from the apex downward, and the endospore, dilated with fluid, protrudes through the gap (*figs. 98, 99*). The sperm cells separate from one another, and a single spermatozoid is organized in each. These are spirally coiled like those of *Osmunda*—two complete turns and a part of a third—but I can demonstrate no cilia (*figs. 33, 100*). Neither in appearance nor in movement do they resemble any bryophyte spermatozoid with which I am familiar, or have seen figured. The movement of biciliate gametes is characteristically different from that of an ordinary fern. These spermatozoids progress with a screw-like motion. The latest stages of development occur in the strobili after they are shed.

MICROSPORANGIUM.—At maturity the microsporangium wall consists of two layers of cells, of which the outer is divided into regions of thick and thin-walled cells, which enable the sporangium to open lengthwise into two symmetrical valves (*fig. 62*). The microspores are discharged with much force. If plants that have become somewhat dry are watered copiously and covered with a bell jar, the ripe microsporangia burst open and jerk back their valves, which instantly recoil and hurl the microspores. These may be seen, looking like red powder, lodged in the axils of leaves or upon any other part of the plant where they have chanced to fall. Of necessity the waste must be great. If this is the method adopted by the plant to secure fertilization it may account in a measure for the extremely small number of sporophytes that are developed.

S. apus grows most profusely in a neighboring locality, where almost daily observations have been made during the past year. Early in May 1900, when this work was undertaken, the strobili were well advanced. It was observed that if several eggs in one

strobilus are fertilized, the formation of new sporangia is checked. The sporophylls begin to decay, as finally does the axis immediately below the fruiting head. The production of new strobili went on until late in August, at which time all the strobili were shed, whether fertilized or not. In September vigorous vegetative growth took place during the fall rains, and continued until checked by several days of very cold weather in December. In the latter part of the same month, upon removing the snow that covered the plants to a depth of six inches, they were found to be green and ready to grow at any favorable moment. Large sods were lifted, without disturbing the plants, and brought into the warmth of the greenhouse, whereupon they responded promptly to the blander conditions. After a week, upon gently disentangling the individuals in order not to detach the semi-decayed strobili which were partially covered by the soil, I found several young sporophytes which had thrust their cotyledons and roots through the crevices of the megasporangia. From this it seems safe to assume that an embryo may have two periods of growth separated by one of quiescence, quite comparable to those of seed plants.

SELAGINELLA RUPESTRIS.

There could be hardly a more striking contrast in the external appearance of two closely related plants than exists between these two species of *Selaginella* that are natives of New England. *S. rupestris* is to be found in the most exposed situations, growing on the granite rocks on the mountain sides, wherever there has been enough weathering to insure the deposition of a little soil in the hollows. For six months of the year they endure alternate drought and drenching, and the frequent and rapid changes of temperature which are characteristic of this region, with no more protection than is afforded by their own structural adaptations. The midsummer conditions are even more trying. The plants that were studied for this work grew on bare rocks upon which the sun beats nearly all day from June to September. The fruiting spikes are to be found in profusion at all times of

the year, but, as will develop later, the spores in the main are sterile. Material was fixed first about June 15, 1900, and at intervals of two weeks thereafter until February 1901. Sods were lifted from the rocks, and planted in shallow boxes which were kept in a warm place exposed to the direct sunlight. The spores were shed freely all summer. I collected other plants from a similar xerophytic situation at Starved rock, Illinois, in August, and for comparison material was sent me in Northampton, Mass., from Austin, Texas, in November. These three regions are 800, 900, and 1600 miles apart. The plants collected in Massachusetts and Illinois, from June until February, almost without exception produced megasporangia only. The Texas material collected in November, on the other hand, was almost purely microsporangiate.² Expecting to find prothallia and young sporophytes developing in the spores that had been shed, these were picked out from the loam from which the plants were growing and killed at frequent intervals during the summer, with the purpose of getting all important stages. In all cases these proved to be barren. Late in August—as was the case with *S. apus*—the young sporophytes were discovered protruding from a withered strobilus that was nearly covered by the dirt. Further search produced many well-developed embryos, but it was apparently too late in the season to secure early stages (*fig. 126*).

During the first week in January, a sudden thaw was succeeded by several days of rain. Plants were collected from the mountain and gradually brought into a greenhouse temperature. The old strobili became greener at their tips, and new vegetative shoots started from the lower part of the stems. It is doubtful whether the increase in the number of individual plants in a given locality is due to any considerable extent to sexual reproduction. A hollow in the rock, or a crevice, becomes filled with a closely compacted colony that is the result in great measure of vegetative reproduction, where prostrate branches, under

² Some of the Texas material was submitted to Professor L. M. Underwood, of Columbia University, for identification. He informs me that it is not *S. rupestris*, but a closely allied unnamed species.

favorable conditions of moisture, have rooted, and later have severed their connection with the parent plant. In examining many colonies, very few young plants that originate from spores are in evidence, although the soil may be thickly beset with the spores that have been shed. Bits of the old plants that have been torn off by the action of the wind or rain are frequently caught in crevices along the precipices, and it is from these that new clumps are most frequently started.

In *fig. 124* I have endeavored to convey an idea of the structural adaptation of the strobilus to its austere environment. The closely overlapping sporophylls form four rows, in whose axils the sporangia have little space to develop. The growing apex is protected by at least twelve and frequently sixteen sporophylls which envelop it. The epidermis is two or three layers of cells thick on both surfaces of the sporophyll, except in a shallow groove running lengthwise along the middle of the ventral surface. In this groove are the comparatively large crowded stomata, which are protected by the next older overlapping sporophyll of the same row. While the leaves are yet very small the apex ceases to grow and becomes transformed into a branched spine. The formation of the horny epidermis proceeds from the apex toward the base. Between the ligule and the sporangium the tissue retains its meristematic nature. The vascular bundle occupies the central shaft of the strobilus, and is augmented from the apex downward by the leaf traces that join it from the sporophylls. Four large communicating air spaces, spanned by trabeculae, surround the entire system. These communicate with the exterior by certain larger chambers into which the stomata open. There is little closely compacted tissue in either sporophyll or stem. All the cells except the epidermis and the vessels contain chlorophyll. This structure is in marked contrast to the delicate unprotected sporophylls of *S. apus*.

A single superficial cell, which uniformly is so close to the base of the subtending sporophyll that it is impossible to determine whether it belongs to the stem or the leaf, is the origin of

the sporangium (*fig. 101*). The sporophyll immediately above it starts simultaneously from an exactly similar cell. A wall separates the archesporial cell into an epidermal cap cell and a hypodermal cell (*fig. 102*). The sporangium wall develops from the former, the sporogenous tissue from the latter. A few sporogenous cells near the base originate from the stem tissue (*fig. 105*).

The tapetum and sporangium development are quite like those of *S. apus*. The sporophylls and stem apex, on the other hand, have no apical cell. The ligule appears later in the history of the sporophyll than in *S. apus*, and the megaspore mother cell is somewhat larger. Certain cytological features serve to distinguish these cells from the sterile sporogenous cells (*fig. 106*). The division into spores presents some curious variations. Sometimes the spore mother cell divides once only, forming two megaspores; again, after the first division, one of the daughter nuclei may divide, or occasionally both. In any case, so far as observed, but one or two normal megaspores are ever formed; if there are others, they are dwarfed in size and never grow. That the exospore is a membrane common to both spores is evident from *figs. 119, 120, 121*. The formation of the mesospore and endospore is not preceded by any such deeply staining layer as is represented in *fig. 42*. The protoplasmic vesicle expands and overtakes the spore wall. This stage terminates the development of the megaspore (*fig. 123*). In the older sporangia, nearer the base of the strobilus in the specimens that I examined, no further development had occurred beyond increase in size and thickness of the spore coats—the contents showed that the spores were abnormal.

This sequence of events, although followed step by step repeatedly, had been rejected as probably not normal, until the sporophytes were discovered. As the number and shape of the megaspores in these sporangia which contained embryos agree with the stages described, in spite of the gap that remains to be bridged between the megaspore and mature gametophyte, it seems reasonable that the observations can be relied upon as far as

they go (*figs. 108-120*). A very close series of stages from start to finish is necessary to produce confidence in any interpretation of phases of a plant which displays so many irregularities, and evidently has so nearly lost its power of sexual reproduction.

The development of the microspores has not yet been followed in detail. Observations on this subject, together with the growth of the female gametophyte and the embryology, will be given in a future paper. One interesting feature that may be noted now is the incomplete septum which projects into the cavity of the microsporangium (*fig. 125*). This is formed in part from sterilized sporogenous tissue and in part from the cells of the pedicel.

Since the above was written, a package of fresh material has been sent me from Texas. All the plants have begun their spring growth and the winter strobili are growing at the apex. The first sporophylls of the season subtend megasporangia. I find not more than eight or ten in each strobilus. Above these are microsporangia to the number of twenty or thirty. In this material it has been my good fortune to observe the method by which the spermatozoids obtain their entrance to the megasporangia. A perfectly fresh, vigorous strobilus was cut from the plant and stripped of its sporophylls, thus exposing the sporangia *in situ*. A megasporangium, which was observed gaping open and in close proximity to a microsporangium that had discharged its microspores, was separated from the strobilus and examined under the microscope. Six microspores were caught on the sculptured surface of the megasporangium near the edge of the valves, which were slightly separated from each other. One microspore, evidently mature from the fact that the exospore was split and the endospore protruding, suddenly discharged a current of slime which at first proceeded directly away from the megasporangium. Soon, however, as if acted upon by some attractive influence from the gametophytes within, the stream turned abruptly and entered the opening between the megasporangium valves. The spermatozoids were swimming with characteristic rotary motion in this stream. With difficulty, it was

possible to make out that they were similar in shape to those of *S. apus*, but much smaller. To each was attached a vesicle. An attempt to stain them with osmic acid on the slide failed to demonstrate any more details.

ADDENDUM.—Since the foregoing was forwarded for publication it has been possible to verify the main facts and to explain certain apparently contradictory features with regard to *S. rupestris*. The spring and early summer of 1901 were extremely wet as compared with those seasons in 1900. *S. apus* growing in low pastures made few strobili and aborted spores in contrast to the profusion of the year before, whereas *S. rupestris* thrived in the unusual supply of moisture and developed strobili, which in turn produced embryos prodigally. The impotency of the megaspores gathered in midsummer of 1900 was obviously due to the lack of water; for at the expiration of the rainy season this year, the last of June, and before the plants had suffered from drouth, they were lifted with all the underlying humus and placed in an open situation in the Botanic Garden. Profuse watering was continued and thorough draining secured. A large percentage of the strobili ripened after fertilization had occurred, and instead of few embryos found with much effort as in the season of 1900, hundreds of young sporophytes have been secured. The sequence of events in a propitious season, based upon the observation of two years, seems in brief to be this: Strobili are formed on the new vegetative shoots of the plant in late summer and autumn. Only megasporangia develop that season, and in these the gametophytes reached the stage bearing archegonia. In the spring, these strobili resume their apical growth, and first microsporangia appear. Thus each strobilus has a basal zone of megasporangia approximately six months old, and above it a narrow region of microsporangia. The number of microsporangia appears to be strictly limited. I have found eight to twelve as a rule. Thenceforth so long as the strobilus continues to grow during the remainder of that season megasporangia only are developed. The production of these is checked in case embryos form in the lowermost zone of

megasporangia. Immediately upon the cessation of growth in the strobilus and during its ripening, a vegetative lateral bud on the axis immediately below the strobilus is stimulated into activity, develops horizontal branches, and roots which grow down into the humus. When this new growth is thus made independent, the axis bearing the strobilus and the germinating embryos decays, and at the period when cotyledons and roots of the young sporophytes are thrust out of the sporangia, the strobilus is lying in contact with the ground, shaded and otherwise protected by the vigorous vegetative growth that is spread above them. The strobilus decays much more slowly than the leafy axis beneath. This accounts for my finding strobili which appear to be shed from the plants. In case a strobilus fails to produce embryos, either through failure in fertilization, or probably more often because the dry season overtakes the gametophyte at a critical stage of its growth, the strobili continue to grow indeterminately throughout the season, and apparently perform the vegetative work that, under more auspicious conditions, is taken up by the new growth from the lateral bud. Moreover, the spores rendered sterile by adverse conditions are shed from the sporangia. Thus not infrequently one finds greatly elongated strobili with a basal zone of empty gaping sporangia, surmounted by two or three whorls of empty microsporangia, then a zone of sprouting embryos, and an apical region of degenerating young stages of megasporangia. In contrast with this are the strobili which have developed under continuous favorable conditions, in which the *basal zone* displays embryos. Whether or not the strobilus may carry on its growth a third year in case of two successive failures to produce embryos, remains to be demonstrated. It will be a matter of interest to discover what variation in ecological conditions causes the strobili of the allied Texas species to pass the winter in a microsporangiate condition, whereas *S. rupestris* in Massachusetts appears to bear during the same season only megasporangia.

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[To be continued.]

A STUDY OF THE SPORANGIA AND GAMETOPHYTES
OF SELAGINELLA APUS AND SELAGINELLA
RUPESTRIS.

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY.
XXXI.

FLORENCE MAY LYON.

(WITH PLATES V-IX)

[Concluded from p. 141.]

GENERAL DISCUSSION.

CONSIDERING the phylogenetic importance of the group, the literature on the Selaginellaceae is surprisingly scanty. More especially is this true of that which treats of the development of spore and gametophyte. Some marked discrepancies in essential features have appeared in these papers, however, and show how much detailed life histories of more species are needed before further inferences are drawn as regards the relation of this group to others. Only one comparatively full account of a single species has yet appeared, Bruchmann's monograph on *S. spinulosa*, published in 1897, although he gives no account whatever of the development of the male gametophyte nor of the sporangia. He had much difficulty in obtaining normal spores,¹ and therefore was unable to follow the earliest stages of the prothallium in detail. In general, however, he agrees with Heinsen and Arnoldi that there is free cell formation within the spore, followed by periclinal and anticlinal cell walls, thus making a disk-shaped mass of cells in the apical portion of the

¹"Über die Keimung der Sporen habe ich zu zwei verschiedenen Malen Versuche angestellt. Das erste Mal sate ich sie gleich nach ihrer Reife (im August) auf Torf aus, hielt sie beständig feucht und schützte sie im Winter gegen Frost. Die erste Keimung weniger Sporen dieser Aussaat bemerkte ich erst nach zwei Jahren, weitere keimten dann in dritten Jahre, aber der grosste Teil zeigte selbst nach solcher Zeit keine Keimung."

spore. The upper layers of cells are much smaller and irregularly polyhedral in shape. No diaphragm is formed. Three tufts of trichomes grow from the upper surface of the prothallium, rend the exospore into three flaps along the ridges at the apex, and push them up out of the way of the developing archegonia. Only a limited number of the latter develop, some five to ten as compared with Pfeffer's report of thirty in *S. Martensii*. There are four rows of neck cells, with three cells in each row. Pfeffer reports but two cells in each of the four rows in *S. Martensii*; Campbell reports two in *S. Kraussiana*. Heinsen gives a list of eleven species: *S. Martensii*, *S. lepidophylla*, *S. Willdenoviana*, *S. denticulata*, *S. apus*, *S. erythropus*, *S. Helvetica*, *S. serpens*, *S. Douglasii*, *S. glauca*, *S. pilifera*, and says that he agrees with Pfeffer on this point. Bruchmann finds that although several embryos may start to grow, but one comes to maturity. The first division of the oospore of *S. spinulosa* is transverse to the axis of the archegonium, and the cell nearer the neck becomes a suspensor.

The most notable fact, however, is that this embryo has no foot. "Ein Fuss in dem Sinne, sie ihn z. B. *S. Martensii*, *S. Kraussiana* und andere Arten besitzen, fehlt." Whereas, the embryo of *S. apus*, as I have found, has no suspensor. After fertilization the cover cells of the archegonium close together, the walls of the neck cells thicken and turn brown. On the other hand, Pfeffer maintains that the neck of the archegonium of *S. Martensii* gapes widely during the entire embryonic development; and he represents the suspensor pushing up into the neck canal like a wedge. This latter method is the rule in most pteridophytes, and hence *S. spinulosa* together with *S. apus* and *S. rupestris* are exceptions.

In the last twenty years, as far as I know, but four other contributions have been made to a knowledge of these phases of the history of the Selaginellas. In 1871, Pfeffer published his paper "Die Entwicklung des Keimes der Gattung Selaginella." The extreme difficulty of interpreting the spore and prothallial structures, even with the aid of all the modern technique, makes

the description given by this author seem an extraordinary performance, and none the less that apparent discrepancies with other species may disappear upon reexamination. His account of *S. Martensii* differs from the foregoing in the following particulars: The megaspore when about one fourth its final size possesses two coats, exine and intine. Against the inner surface of the latter lies a thick layer of protoplasm inclosing a transparent fluid in which floats a large "nucleus." Near the apex "the protoplasm has a different appearance," but he was unable to interpret it. Obviously, he misinterpreted the so-called nucleus, which is the protoplasmic vesicle, and in all probability the peculiar appearance near the apex was due to the presence of the real nucleus. A dome-shaped diaphragm separates the gametophyte into two regions. This he thinks arises at the first division of the spore into two cells. The one toward the apex of the spore becomes subdivided into a tissue three layers thick in the middle, one layer at the periphery. The continuous division of the lower cell fills the basal portion of the spore with larger cells. The hollow spherical portion of the gametophyte below the disk he describes as becoming filled with "freigebildete Primordialzellen." He could not demonstrate the presence of nuclei in these cells, but regards it as probable. According to his statement, the growth of the prothallium occurs at two periods. The disk-shaped mass of cells appears before the spores are shed, the tissue below the diaphragm, the "secondary endosperm," afterward. Archegonia do not develop until the spores fall.

In 1894 Heinsen reexamined the species studied by Pfeffer. He fell into the same error as regards the interpretation of the protoplasmic vesicle and nucleus in the young megaspore. He was unable to determine the origin of the megaspores, other than that a tetrad arose endogenously in the spore mother cell. He denied the existence of a primary and secondary endosperm, and of a diaphragm, and corrected Pfeffer's misinterpretation of the food balls in the gametophyte cavity "as freely formed cells." Infrequently he found the archegonia formed in unshed spores:

The diaphragm which Pfeffer thought was the wall of the first division of the spore Heinsen regards as the plane of separation between the small cells at the apex of the gametophyte and the larger ones below, and not a wall. He also refutes Pfeffer's statement that ultimately the cell divisions completely occupy the basal region of the spore. Heinsen lays great stress on the supposed fact that the nuclei of the Selaginellaceae increase solely by direct division. He investigated material killed at all hours of the day and night, not only spores but vegetative tips, with special reference to establishing this point. He found a total absence of karyokinetic figures. As this statement, which if true would be most surprising, has not been refuted by later writers, it may be of interest to note a possible explanation of the error. In describing his methods of imbedding, Heinsen says that he killed his material by immersing some specimens for ten minutes in Flemming's fluid, others three minutes in a 1 per cent. corrosive sublimate solution, still others "mit gleich gutem Erfolge warte ich ein zweimaliges schnelles Eintauchen in kochendes Wasser an." It is doubtful whether any of the killing fluids thus employed had opportunity to penetrate the sporangium wall in so brief a contact; in which case his material died a lingering death through the washing and dehydrating processes. Under such conditions naturally there would be no traces of karyokinesis. The statement that he had as good results from two quick dips in boiling water as from the Flemming fluid is otherwise inexplicable. It is somewhat difficult to determine whether Heinsen means his statements to be general with regard to all the species he enumerates or not. His summary leads one to assume that the sequence of events there set down is true of the eleven species above named. There are certainly inaccuracies in many details if applied to *S. apus*.

Arnoldi in the *Botanische Zeitung* of 1896 followed in a brief article on "Die Entwicklung des weiblichen Vorkeimes bei den heterosporen Lycopodiaceen." He investigated *S. cuspidata elongata*, and gives a résumé of Heinsen's paper. He agrees with Heinsen on all points, thus reiterating the misinterpretation of

the spore content. Campbell in his *Mosses and Ferns*, published in 1895, gives an account of his investigation of *S. Kraussiana*. He too describes "the single large globular nucleus" (*i. e.*, the protoplasmic vesicle) of the megaspore. The diaphragm which exists in this species he explains as arising, not as Pfeffer thought from the first division of the spore, but from the thickening of the walls of the lowermost layer of cells of the disk at the spore apex. He finds "numerous small nuclei" scattered through the protoplasm of the spore cavity, and the protoplasmic layer thickens until it "completely fills the cavity of the spore." I have examined *S. Kraussiana* with reference to these points. The diaphragm is very evident and obviously formed in the manner described by Campbell. I can demonstrate no nuclei at any period in the spore cavity, nor any protoplasm. The protoplasm forms a layer next the wall, as in *S. apus*, and the vacuole within is full of food matter, at first a fluid, then an emulsion, and finally filled with granules and small balls of matter which stain like nuclei. The protoplasmic layer grows thicker, but never fully occupies the interior space as Campbell describes.

The latest contribution is that of Fitting (1900), "Bau und Entwicklungsgeschichte der Makrosporen von *Isoëtes* und *Selaginella* und ihre Bedeutung für die Kenntnis des Wachstums pflanzlicher Zellmembranen." He corrects the error made by each of the above named writers in interpreting the parts of the megaspore. An attempt to discover the exact origin of the megaspores failed, due to the fact that the spore mother cell stained so deeply in all his preparations that the details of the evolution of the four spores could not be made out. He was unable to avoid shrinkage of cells. There follows a very detailed account of the development of the spore coats, in which he differs from Heinsen as regards their origin. He drew conclusions largely from living material examined in a salt solution. As I have said before, the interpretation of the origin of the spore coats seems to me largely a matter of theory, and one who has examined the young spores of *S. apus*, while inclosed in the sporangium and surrounded by sterile mother cells, is

skeptical of the results obtained by this method of investigation. He fails to comment on Heinsen's statement with regard to the method of cell division. Both Arnoldi's and Fitting's articles have been comparative studies of certain phases of the Selaginella life history with the same stages of Isoëtes. Thus, until the year 1900 we have not had a correct interpretation even of the parts of the megaspore.

MALE GAMETOPHYTE.—We are indebted to Millardet's memoir for our earliest knowledge of the male gametophyte. This work appeared in 1869, and almost no detail of importance has been added by later workers. In 1885 Belajeff repeated Millardet's work on the same species and corroborated all essential details. There has been no other during the last sixteen years until the present account given in this paper of *S. apus*. How much the discrepancies between the two are due to differences in methods of technique, rather than to specific characteristics, remains to be demonstrated. Millardet and Belajeff both examined the microspores of several species of Selaginella (*S. Kraussiana*, *stolonifera*, and *cuspidata*) in living condition, then added various reagents to the microscope slide, focusing through the spore coats or crushing them by pressure of the cover glass, to determine the phenomena taking place within. The material for the account of the male gametophyte of *S. apus* was killed, imbedded, sectioned, and stained without removing the spores from the strobilus. It will be seen that the main difference lies in the fact that in the earlier accounts there are eight cells described which constitute an antheridial wall; that later these cells disappear, and the sperm cell complex floats free in the cavity thus formed. Both authors state that they were unable to secure a cellulose reaction for the cell walls. On the other hand, I could demonstrate the presence of no such walls in *S. apus*. The first division of the spore results in two free cells. The first, according to Pfeffer's and Millardet's view, is the reduced vegetative part of the prothallium. The latter, which I have called the generative cell, divides at once into a complex of sperm cells. Is it possible that the protoplasmic films surrounding the vacuoles

containing food bodies were mistaken by these authors for cell walls? It is not quite certain that the species of *Selaginella* called *Kraussiana* in America is the identical one known by that name in Europe. The writer investigated the American form and found the sequence of events identical with those that obtain in *S. apus*. In attempting to repeat the methods employed by Millardet and Belajeff, it was impossible to distinguish through the spore wall the nuclei from the food granules, as both stained alike. Removing the exospore by shoving the cover glass to and fro was a tedious performance, whose results hardly repaid the effort. Moreover, it was impossible to manipulate the stains with enough precision to differentiate the nucleoli. As regards the spermatozoids, explicit statements are few. Belajeff figures those of *S. cuspidata* as biciliate bodies, somewhat elliptical in shape, pointed at the ends, with a slight spiral twist. Millardet gives substantially the same description, but no figures. In connection with his account he makes this reference to the work of M. Roze, who had published his observations in 1864:

Ainsi qu'on le voit, mes observations sur la forme des anthérozoïdes du *Selaginella Kraussiana* sont loin de s'accorder avec celles de M. Roze sur les anthérozoïdes des *S. Martensii* et *S. Galeottii*. Toutefois, comme il a en affaire à des espèces différentes de celle que j'ai étudiée, je m'abstiens de toute discussion sur ce sujet.

That there existed little information on the subject at this time (1869) is evidenced by the following statement:

Les seules observations que je connaisse sur la germination des microspores du genre *Selaginella* sont celles de M. Hofmeister et de M. Roze. Ces deux auteurs se sont bornés à mentionner le fait de la production, dans la spore, de cellules contenant chacune un anthérozoïde. C'est M. Hofmeister qui a constaté le premier l'existence, et la de ces animalcules; M. Roze a montré qu'ils sont biciliés.

So far as I can determine, the authors who have described spermatozoids of named species of *Selaginella* that they themselves have seen are as follows:

1862. Hofmeister.

1864. Roze first demonstrated two cilia in *S. Martensii* and *S. Galeottii*.

1869. Millardet thus describes the spermatozoids of *S. Kraussiana*:

Il m'a semblé qu'elle se colore en violet par le chlorure de zinc iodé; je n'ose toutefois l'affirmer. On voit en dedans d'elle un filament roulé en béliçe, soit à droite, soit à gauche, de façon à faire environ deux tours complets; l'une de ses extrémités est occupée par le granule réfringent d'amidon que je viens de signaler et par suite extrêmement visible; l'autre est à peine distincte. La première constitue la partie postérieure du corps de l'anthérozoïde, elle décrit un cercle plus étroit que le reste de l'animalcule; on voit d'habitude à côté d'elle quelques granulations à peine appréciables. Les cils semblent placés l'un à côté de l'autre; on les distingue difficilement du corps.

. . . Ces détails serviront à l'intelligence des différentes formes des spermatozoïdes. Dans leur état de développement complet, ils sont entièrement débarrassés de leur vésicule et de la membrane de leur cellule mère, et présentent à l'extrémité postérieure un corpuscule d'amidon; il est resté dans la vésicule où on le retrouve; c'est là une seconde forme. Une troisième est constituée par les animalcules qui portent avec eux une vésicule; une quatrième, par ceux qui ne sont qu'incomplètement dégagés de leur cellule mère.

Dans la forme la plus développée, le corps est presque droit et décrit une spirale à peine sensible. Sa longueur dans cet état est 0.018 mm environ. Il s'amincit graduellement depuis l'extrémité postérieure jusqu'à la naissance des cils: son épaisseur maximum ne dépasse pas 0.0007 mm environ. En avant il se bifurque et se termine ainsi par deux cils très-tenus, deux fois aussi longs le corps.

1871. Pfeffer described the male gametes of *S. Martensii*, and *S. caulescens*.

1885. Belajeff examined *S. cuspidata*, *S. laetevirens*, *S. Martensii*, *S. caulescens*, *S. stolonifera*, *S. Kraussiana*, and *S. Poulteri*. Of these he gives figures only of the spermatozoids of *S. stolonifera*.

Thus, in the thirty-nine years since the spermatozoids of the Selaginellaceae were first described, there have been only four contributions upon the subject in which the spermatozoids of but eight species are described from direct observation vouched for by the writer.³ The difficulty of determining

³ 1. *S. Martensii*: Roze, 1864; Pfeffer, 1871; Belajeff, 1885.

2. *S. Galeottii*: Roze, 1864.

3. *S. Kraussiana*: Millardet, 1869; Belajeff, 1885.

4. *S. caulescens*: Pfeffer, 1871; Belajeff, 1885.

5. *S. cuspidata*: Belajeff, 1885.

6. *S. laetevirens*: Belajeff, 1885.

7. *S. stolonifera*: Belajeff, 1885.

8. *S. Poulteri*: Belajeff, 1885.

the structure of bodies so extremely small can hardly be over-estimated.

With either a Bausch & Lomb $\frac{1}{15}$ oil immersion objective and $\frac{3}{4}$ ocular, or a Zeiss oil immersion 2^{mm} and ocular 3, I was able to make out the spermatozoids only by their characteristic rotary movements as they left the microspore of *S. rupestris*. Their spiral form and attached vesicles were facts determined rather by interpreting appearances by those I had definitely seen in apparently similar but larger bodies, than by actual observations. The spermatozoids of *S. apus* are somewhat larger, and I feel that there is less likelihood of error in describing them. It is obvious that a more critical examination of many species is needed before much weight be placed upon the so-called aberrant forms of the Selaginella spermatozoids in tracing the phylogeny of the group.

THE DEVELOPMENT OF THE SPORANGIA.—The two most recent and important papers on the development of the sporangium are Goebel's (1880) and Bower's (1894). I shall quote verbatim Bower's summary of results from Selaginella :

1. The sporangium is eusporangiate, and arises from the tissue of the axis, above the subtending leaf ; the position varies in different species.
2. The origin of the sporangium is similar to that of Lycopodium, and especially resembles *L. inundatum*, to which species the mature sporangium also is similar in form.
3. Two primary archesporial cells are usually present in each radial section, and these are derived, as in *L. inundatum*, from segmentation of two distinct cell-rows ; as seen in tangential section, the archesporium is referable to three or four such cell-rows.
4. The first periclinal divisions in these cell-rows do not always define the archesporium finally ; subsequent periclinal divisions may result in addition to the central mass, as has been proved for Equisetum ; but here the addition is less regular.
5. The tapetum results from tangential division of the outermost cells of the central mass ; the greater part of it originates as described by Goebel.
6. The tapetum is thus a sterilized part of the potential sporogenous tissue ; a further example of sterilization is seen in the megasporangium, where all the sporogenous cells are disorganized, excepting the one mother cell of the megaspores.

7. Abortive sporangia are to be found at the base of the strobilus as in many species of *Lycopodium*.

With regard to the derivation of the sporogenous tissue, repeatedly radial sections show a distinct plane of segmentation separating the sporogenous tissue into two such definite regions that it is difficult to avoid the conviction that each complex is the progeny of two independent cells. The term "archesporium" is used by Bower to signify the lower cell or cells. I have used the term to indicate the superficial cell itself. With regard to the fourth statement, my observations on *S. apus* do not agree. With the fifth and sixth statements, as regards the origin of the tapetum, my observations are in accord. The seventh statement I find true of *S. rupestris*, but not of *S. apus*.

In no particular do my preparations agree with Campbell's figures of the sporangial development of *S. Kraussiana*.

Of no little interest is the incomplete septum in the microsporangium of *S. rupestris*, which arises above the pedicel. In vertical section, the resemblance to the celebrated fossil *Lepidodendron Braunii*, as figured by Bower (see his *plate 48, fig. 100*), is very marked. He discusses in the accompanying text the possible function of this region of the sporangium. "Possibly," he says, "these extensions of sterile tissue may have facilitated the nutrition of the developing spores, or they may perchance have contributed to the mechanical support of the sporangial wall." In connection with the former theory, it is noteworthy that this septum is found only in the microsporangium, where some of the spores are remote from the tapetum. A feature that may have some bearing on this point is the fact that the megaspore mother cell, so far as I have observed, is always near the periphery of the sporogenous mass and never at the center, a favorable position to secure nutrition from the tapetum. Scott in his *Studies in fossil botany*, 1900, says, *à propos* of this feature in *Lepidostrobus Veltheimianus*, "this structure may be compared with the trabeculae of *Isoëtes*. It is best shown in the microsporangium, but may have originally been present in the megasporangia also."

Of even greater interest is the simultaneous discovery of a paleozoic lycopod each of whose sporangia contains a single megaspore or embryo sac, which presumably was fertilized while still attached to the plant, with the condition that I have described above that obtains in *S. rupestris* and *S. apus*. Of the integument that grows up about the sporangium of the *Cardiocarpon anomalum*, described by Scott, leaving at the top an open slit-like micropyle, there is no trace I think, except that in *S. rupestris*, quite late in the development, after the embryo has formed, the megasporangium becomes sunken in a shallow pit formed by the cushion-like upgrowth of the sporophyll around the pedicel. This outgrowth hedges in the ligule with the sporangium, and may be homologous with the *Lepidostrobus* integument. Scott's statement, therefore, that "the recent discovery of *Lepidostrobi* with integumented, seed-like sporangia, in which only one megaspore came to perfection, shows that some paleozoic members of the group went far beyond any of the living representatives in the differentiation of their reproductive organs," needs modification in view of the fact that *S. rupestris* normally at the present day produces seed-like sporangia with well developed embryos.

SUMMARY.

1. In both *S. apus* and *S. rupestris* the sporangium frequently, if not always, may be traced to a single superficial cell, the archesporium.

2. The sporogenous tissue may arise in two ways. First (*S. rupestris*), from the single hypodermal cell formed by the archesporium being divided by a periclinal wall, thus producing a wall and a sporogenous cell. Second, by the archesporium (which in this case is assumed to consist of two independent superficial cells) dividing into four cells by a periclinal wall, the two hypodermal cells thus formed developing the sporogenous complex. In each case the epidermal cells form the sporangium wall. It is possible but not demonstrated that the second case may be a phase of the first wherein the original superficial archesporial cell divided by an anticlinal wall.

3. The tapetum is formed in part from the sporogenous cells near the exterior of the mass, and in part from adjacent vegetative cells which come to form a more or less regular layer. It is defined very early, and is active and glandular up to the period that the spores have attained their full size, then becomes reduced to a thin epithelial-like layer lying against the sporangium wall.

4. Microsporangia and megasporangia are indistinguishable before the spore mother cells are differentiated. At this stage, in the case of the megasporangium, one or rarely two cells become more conspicuous and divide into spores. If microspores are to be formed, the majority of the cells continue dividing. After the megaspore mother cell is differentiated, all other cells in the megasporangium cease dividing.

5. In *S. apus* four megaspores arranged tetrahedrally are formed within the spore mother cell. In *S. rupestris* four spores may develop in the same manner, only one or two of which may come to maturity; or, most commonly, there may be but a single division of the mother cell nucleus, in which case there are but two spores formed; or again, there may be a redivision of one only of the two daughter nuclei, resulting in three spores of which only one or two attain maturity. Isolated cases have been met with where but one megaspore was formed. As there were no signs of other aborted spores, presumably the megaspore mother cell never divided, but became directly the single megaspore. In no case have more than two spores in a single megasporangium been found which were fertile, and very frequently only one.

6. The megaspore of each species has three distinct coats, the exospore, mesospore, and endospore. The former originates on the inner face of the spore mother cell membrane, and when first distinguishable is a film of unequal thickness. This either directly or indirectly gives rise to the exospore. A thick layer develops between the exospore and the protoplasmic vesicle, which later in its history separates into two layers, the mesospore and the endospore.

7. The female gametophyte is formed by free cell division of the megaspore, the nuclei dividing by indirect division. These nuclei are confined to the apical portion of the spore. Several layers of nuclei are formed by repeated tangential and radial divisions so that there are six or seven in the apical region and one in the basal. Areas of cells are blocked off by protoplasmic radiations passing from the apex outward and inward. The walls of the cells are produced by nuclear plates in the final division. There is no diaphragm, and at no stage of its development are there nuclei in the lower or the central portions of the gametophyte, which at first contains liquid, and finally a semi-solid mass of granular matter.

8. A cushion of cells protrudes through the tripartite cleft in the exospore at the apex. From cells in the upper row of this cushion a limited number of archegonia develop. The cells of this region are markedly smaller than those in the other regions of the gametophyte. No part of the archegonium protrudes from the general level except the cover cells.

9. The megaspores and gametophytes are nourished by matter secreted by the tapetum and passed through the spore mother cell membrane, which persists until the spores are nearly half grown.

10. The microspores develop in a fashion analogous to the megaspore. A large percentage of the mother cells form tetrads, the largest proportion of which are aborted at this stage.

11. The male gametophyte of *S. apus* consists of a single prothallial cell and an ovoid naked mass of potential sperm cells arranged in two groups. There is no antheridium, nor is there a wall which separates the prothallial from the sperm cells.

12. The spermatozoid of *S. apus* is a spirally coiled body; that of *S. rupestris* is of similar shape but much smaller. The presence of cilia in either was not demonstrated.

13. The megasporangia and microsporangia of both species open by definite lines of dehiscence.

14. Fertilization in both species occurs while the spores are

unshed and the sporangia are still attached to the strobilus. At this period the strobilus ceases to form new sporangia. The strobilus of *S. rupestris* retains its physiological connection with the plant until the embryo has produced cotyledons and a root.

15. In the early autumn *S. apus* sheds all strobili whether fertilization has occurred or not. *S. rupestris* retains its strobili throughout the winter and fertilization occurs in the spring.

16. *S. apus* forms twelve to fifteen megasporangia in each strobilus, all of whose spores are generally fertile. The comparatively limited number of embryos formed is due probably either to the limited number of fertile microspores, or to mechanical difficulties in the way of fertilization, possibly both. *S. rupestris* produces strobili far in excess of the number of purely vegetative branches, the majority of which develop only sterile spores except under very favorable conditions. The sterile spores are shed profusely during the summer, and the strobili which remain on the plant throughout the winter retain their power of apical growth. The first sporangia formed in the early spring contain microspores. There is but a limited number of these; then follows under favorable conditions either fertilization or the development of megasporangia, which continues until checked by the development of embryos in the older regions of the strobilus, or in case fertilization fails by the approach of winter.

TECHNIQUE.

The tender tips of the strobili offer no special difficulties in preparation for the microtome, but the older sporangia, especially the closely compacted strobili of *S. rupestris*, are exceedingly resistant to the entrance of reagents, and the hardened epidermis of the sporophyll, combined with the thick walls of sporangia and spores, render sectioning difficult. Certain stages of the spores are peculiarly liable to collapse. These technical difficulties probably explain many gaps in our knowledge. The following method was followed in preparing the material for this investigation.

KILLING AGENTS.—Flemming's weaker fluid, Hermann's chromacetic acid, and bichromate of potassium combined with acetic acid, gave the best results. Corrosive sublimate, absolute alcohol, picric acid, Merkel's and Perenyi's fluid were tried, but the results were unsatisfactory in that I failed to get such successful staining after their use.

These fixing fluids were used boiling hot. In the case of the Flemming, the chromic acid and water were brought to the boiling point, the osmic and acetic acids quickly added and the mixture poured over the strobili which had previously been removed from the plants. The vessel was covered tightly and the contents allowed to cool gradually to a temperature of 30° C. The spores were fixed in twenty-four hours, but often were left in the killing fluid two or three days with no deleterious effects. Water of approximately the same temperature (30° C.) was used for washing, in very large quantities, and changed frequently for two days. Sometimes it was more convenient to use cold running water, in which case three days' washing was necessary to remove all acid. The dehydrating process was equally gradual: twelve hours each in 10 per cent., 20 per cent., 30 per cent., 50 per cent., 75 per cent., and 80 per cent. alcohol respectively. In 95 and 100 per cent. three days each, and the alcohol changed each day. Xylol was added to the specimens with even more caution, as an examination of material from day to day during these manipulations disclosed the fact that at this point danger of collapse was greatest. Six intermediate grades between absolute alcohol and pure xylol were employed, each for twenty-four hours. The strobili remained in pure xylol until they became transparent, which frequently was not for a week, dependent upon the age of the spores. When this condition was obtained, the xylol was replaced by fresh, and small bits of soft paraffin (melting point 30° C.) were added gradually as long as they would dissolve at the temperature of the room. This usually required two days. Thence the vessel was removed to the bath (temperature 40° C.), which was raised slowly to 54° C., soft paraffin being added

during the time up to the point of saturation. At this time the cover was removed from the vessel to facilitate the evaporation of the xylol, and harder paraffin added in small quantities at intervals. At the end of two or three days the temperature was raised to 65° C., and maintained for one week, after which time the xylol had evaporated, and the strobili were infiltrated with paraffin.

At first, on the supposition that such protracted exposure to reagents and heat would injure the more delicate very young sporangia, the tips of strobili were removed, carried through the various media in much less time, and subjected to a temperature of 50° C. for about ten hours only. Comparisons later showed that these precautions were unnecessary, and that the longer exposures produced better results, even in the apical cells. Moreover, having a strobilus cut *in toto* was of the greatest value in interpretation. Further experiments prove that considerable latitude in the direction of longer periods of immersion in the various fluids are not injurious, but all attempts to hasten results by shorter exposure than the period stated above were unfortunate. By an oversight, at one time the temperature rose in the bath three successive nights to 75° C., but with no injurious effects. Some difficulty was experienced in imbedding. The paraffin was inclined to shrink away from the rough surface of the spore wall, which caused the sections to drop out of the paraffin ribbon when transferred to the slide. The difficulty was overcome by removing the strobili to the imbedding dish from the bath, letting them cool off slightly (to about 40° C.), pouring on paraffin at a temperature of 80° C., then cooling as rapidly as possible in ice water. No further trouble was experienced and the strobili could be sectioned without difficulty.

STAINS.—The best stains for the youngest stages of sporangia are iron haematoxylin (Haidenhain method), and the so-called Flemming triple stain—safranin, gentian-violet, and orange G. Frequently gentian-violet and orange G were employed without safranin; for the gametophyte development,

after the appearance of the proteid matter in quantities that obscured the other cell features, cyanin and erythrosin, after treating the sections on the slide with dilute sulfuric acid and chloroform, produced very satisfactory results. The power to take up the stains was retarded in the case of the proteid granules, whereas the nuclei and cell walls were more readily and more brilliantly dyed after this treatment. Karyokinetic figures were especially clear.

It remains my pleasant duty to express my thanks to Dr. John M. Coulter, and to other members of the Botanical Department of the University of Chicago, for many suggestions offered in the course of this investigation. To Mr. E. J. Canning, Head Gardener of the Botanic Gardens of Smith College, I am deeply indebted for aid in collecting and growing material.

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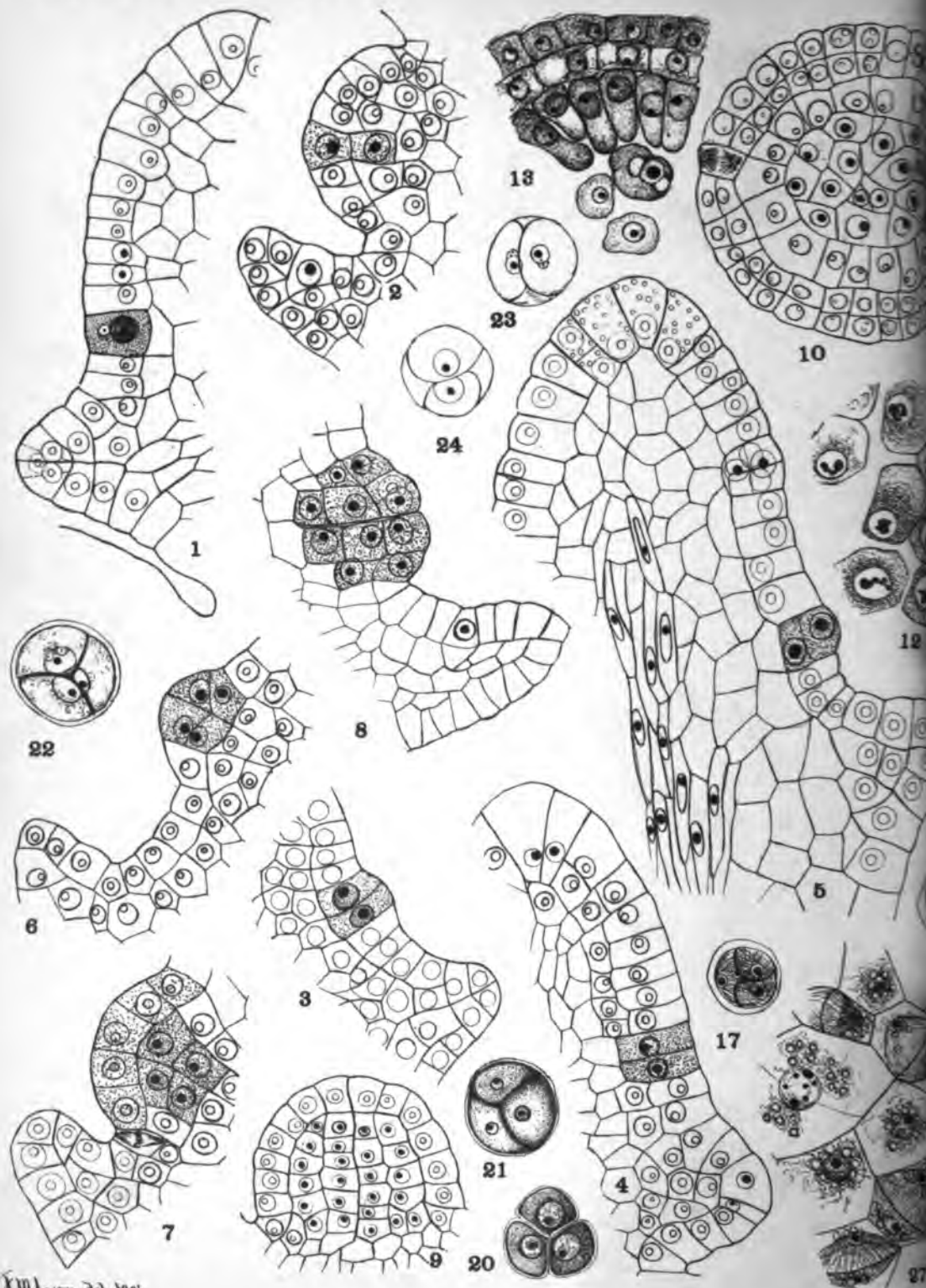
EXPLANATION OF PLATES V-IX.

All figures were drawn with the aid of a camera lucida and a Bausch and Lomb microscope, and were reduced to one fourth their original size in reproduction.

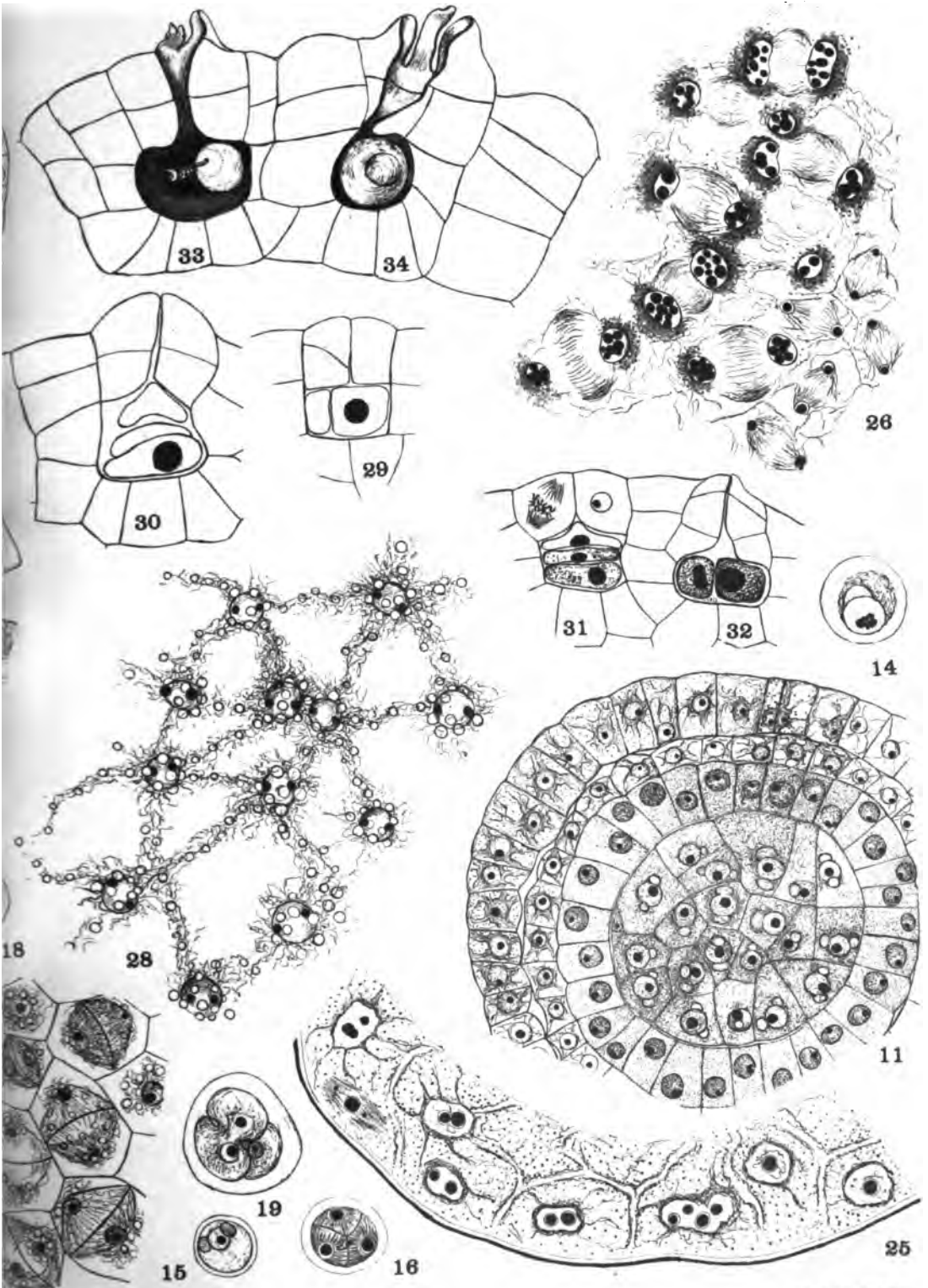
PLATE V. *Selaginella apus*. Oc. $\frac{3}{4}$, oil immersion $\frac{1}{4}$.

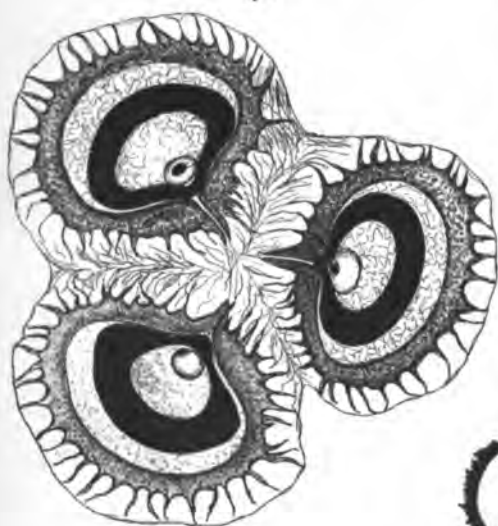
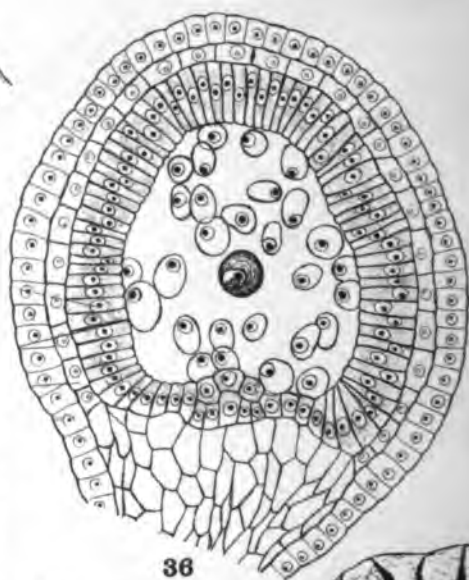
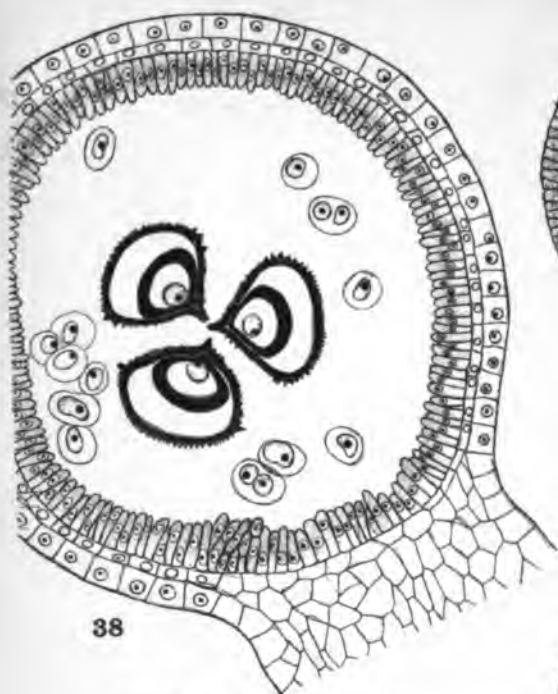
FIG. 1. Vertical median section of tip of strobilus showing the apical cell, a young sporophyll whose apical cell is just established, an archesporial cell (shaded), and a two-celled sporophyll (distinguished by black nucleoli).

FIG. 2. Radial section of portion of strobilus showing sporophyll and two superposed cells (shaded), resulting from a transverse division of the archesporial cell. The superficial cell by repeated division in one plane

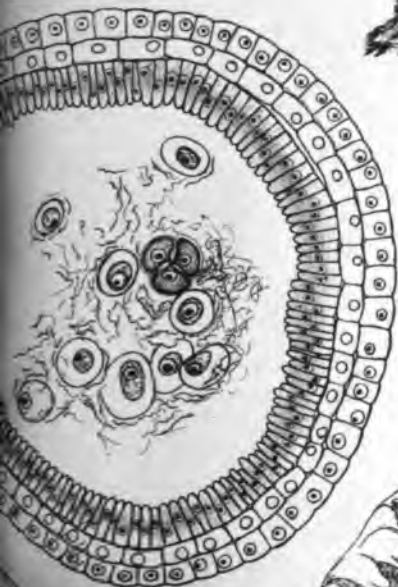


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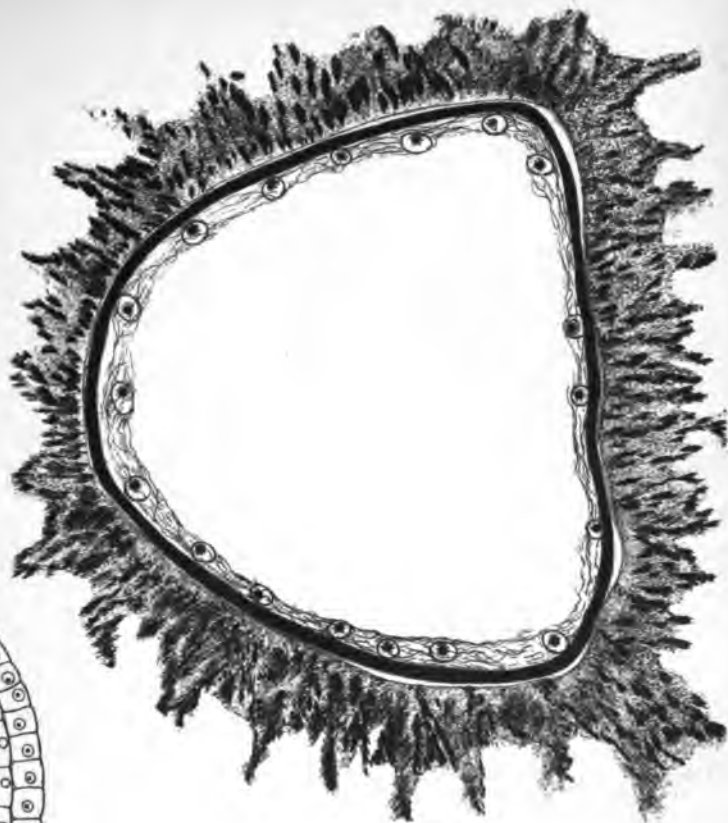
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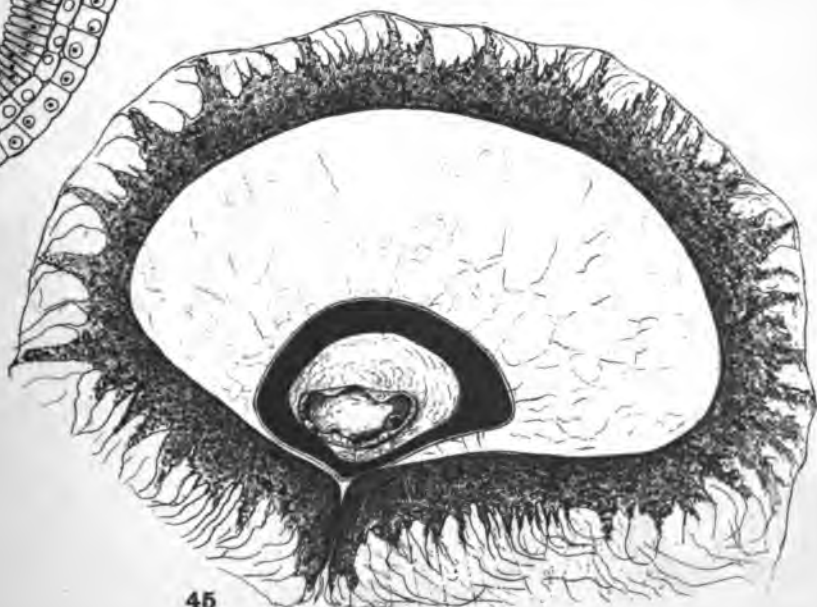
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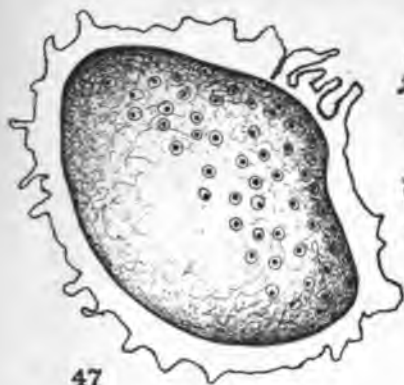
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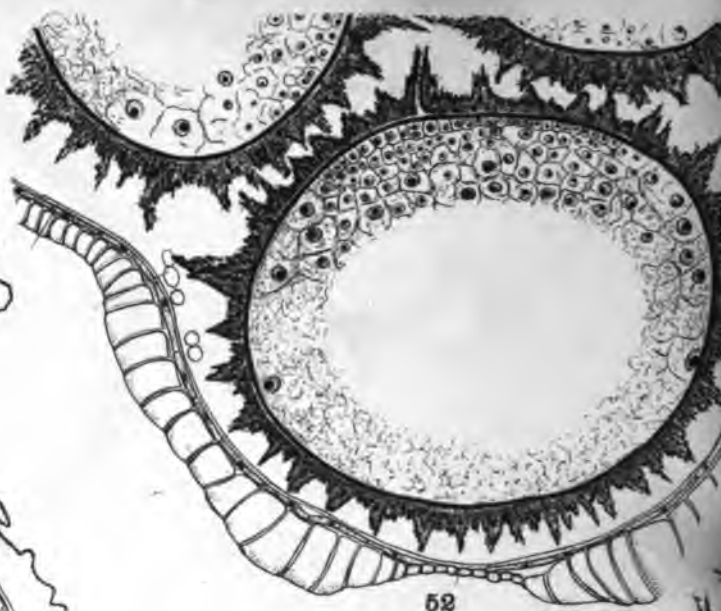
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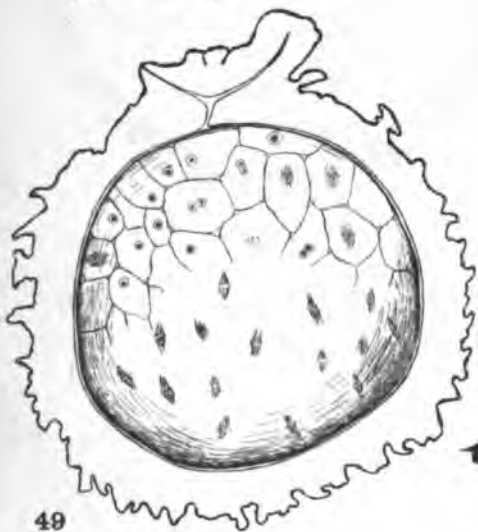
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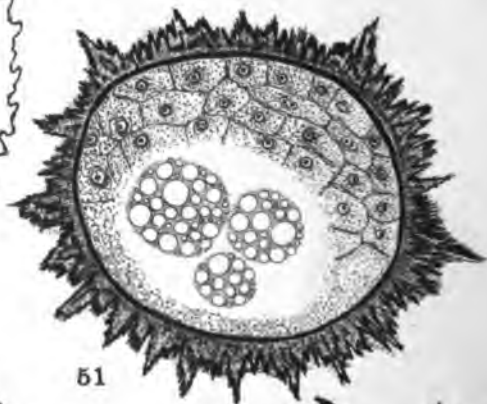
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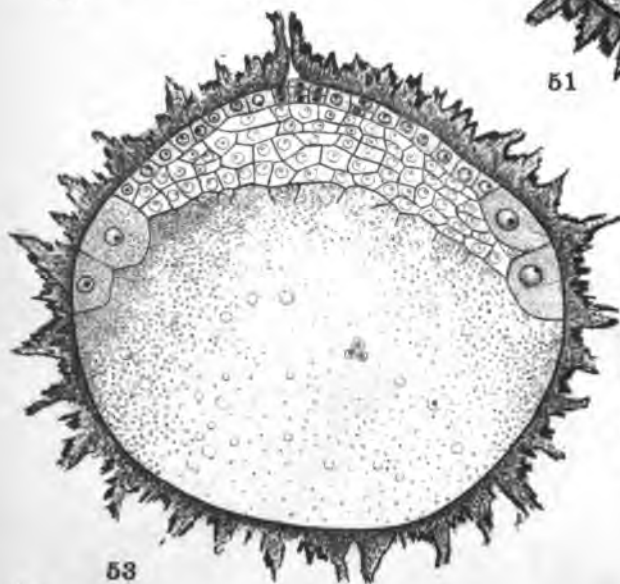
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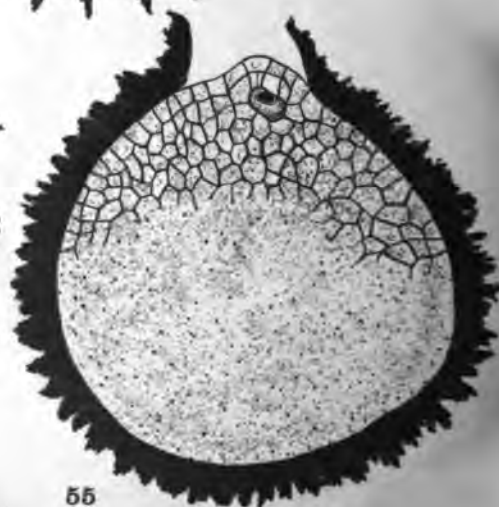
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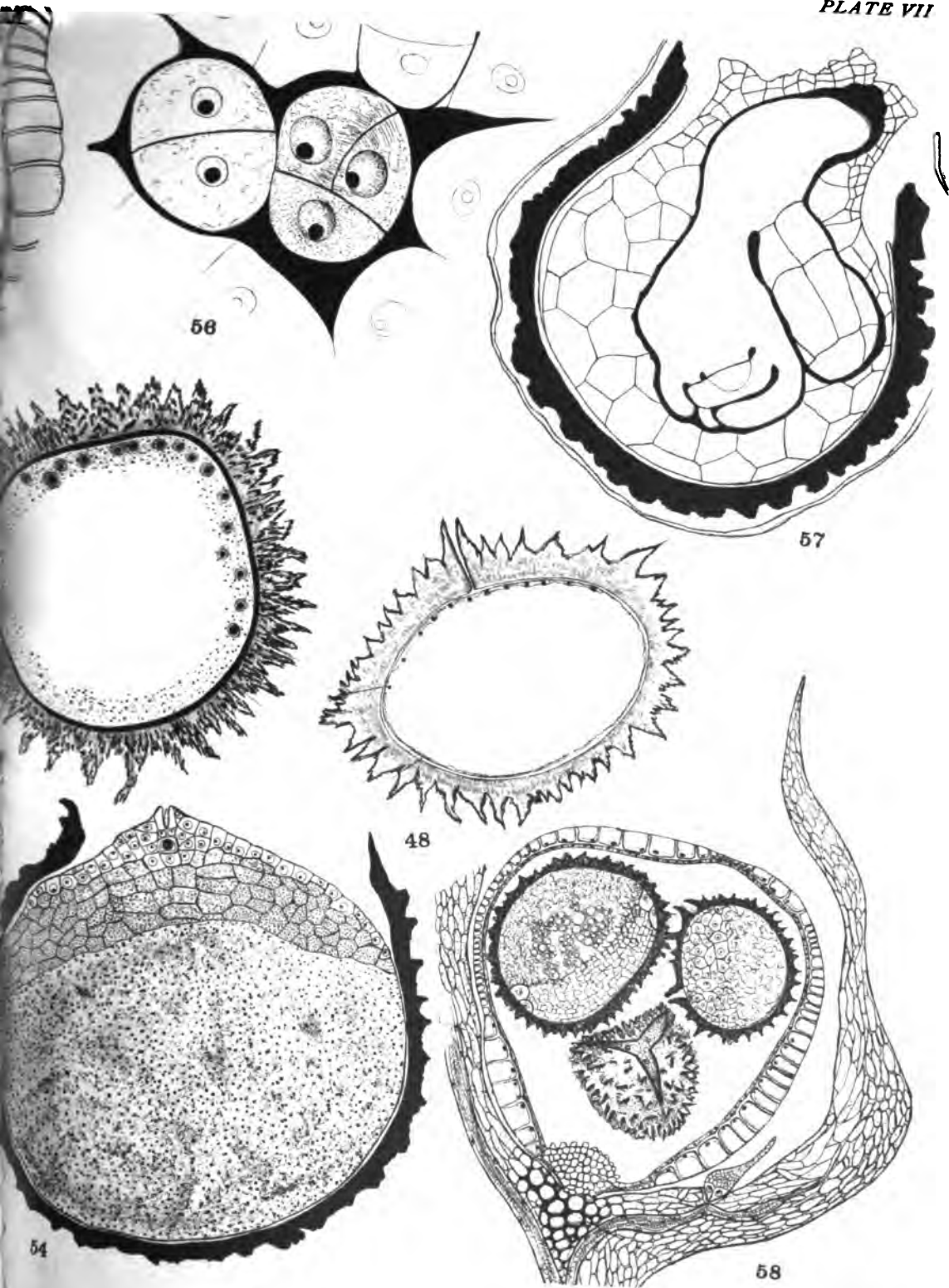
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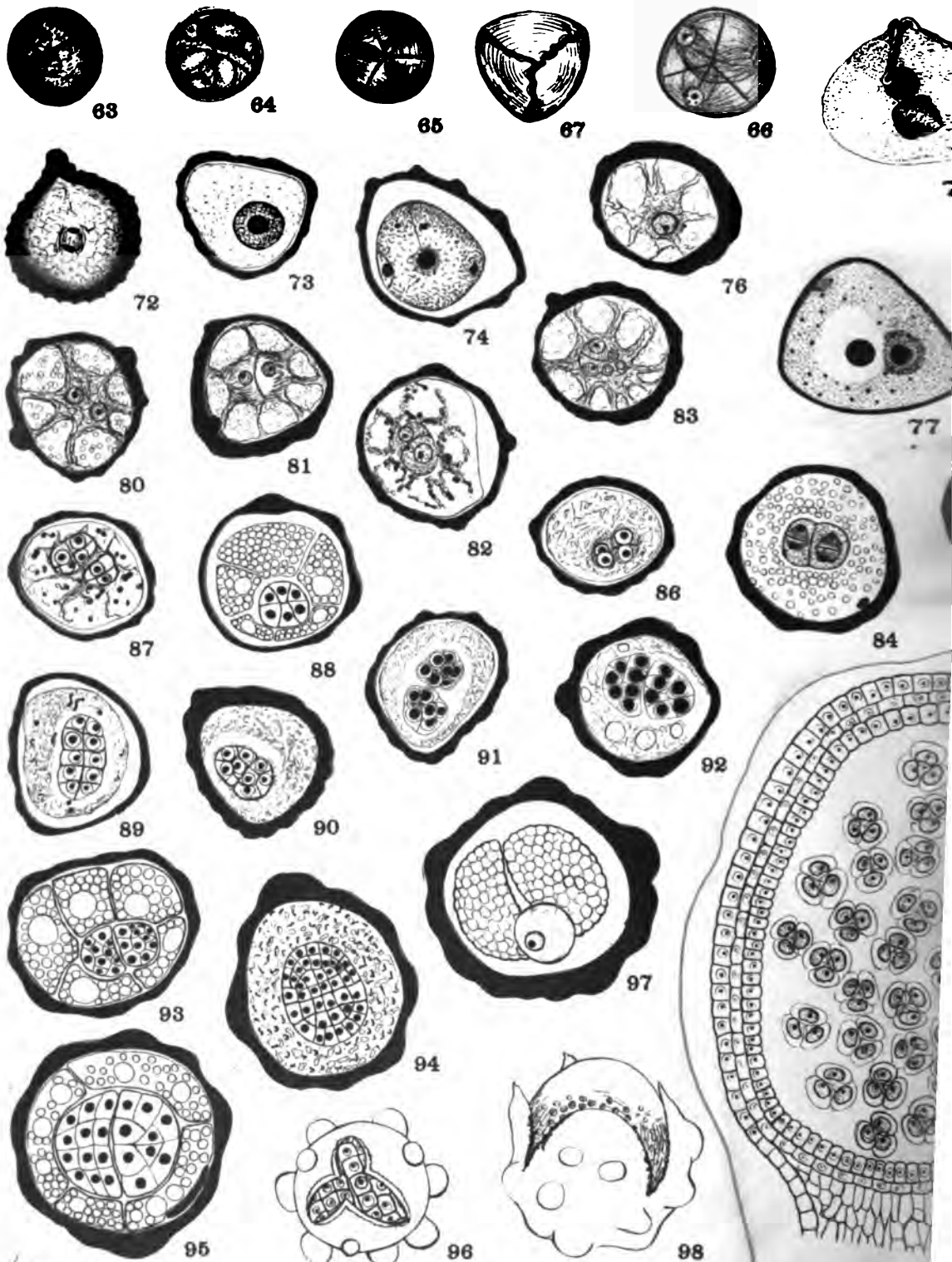


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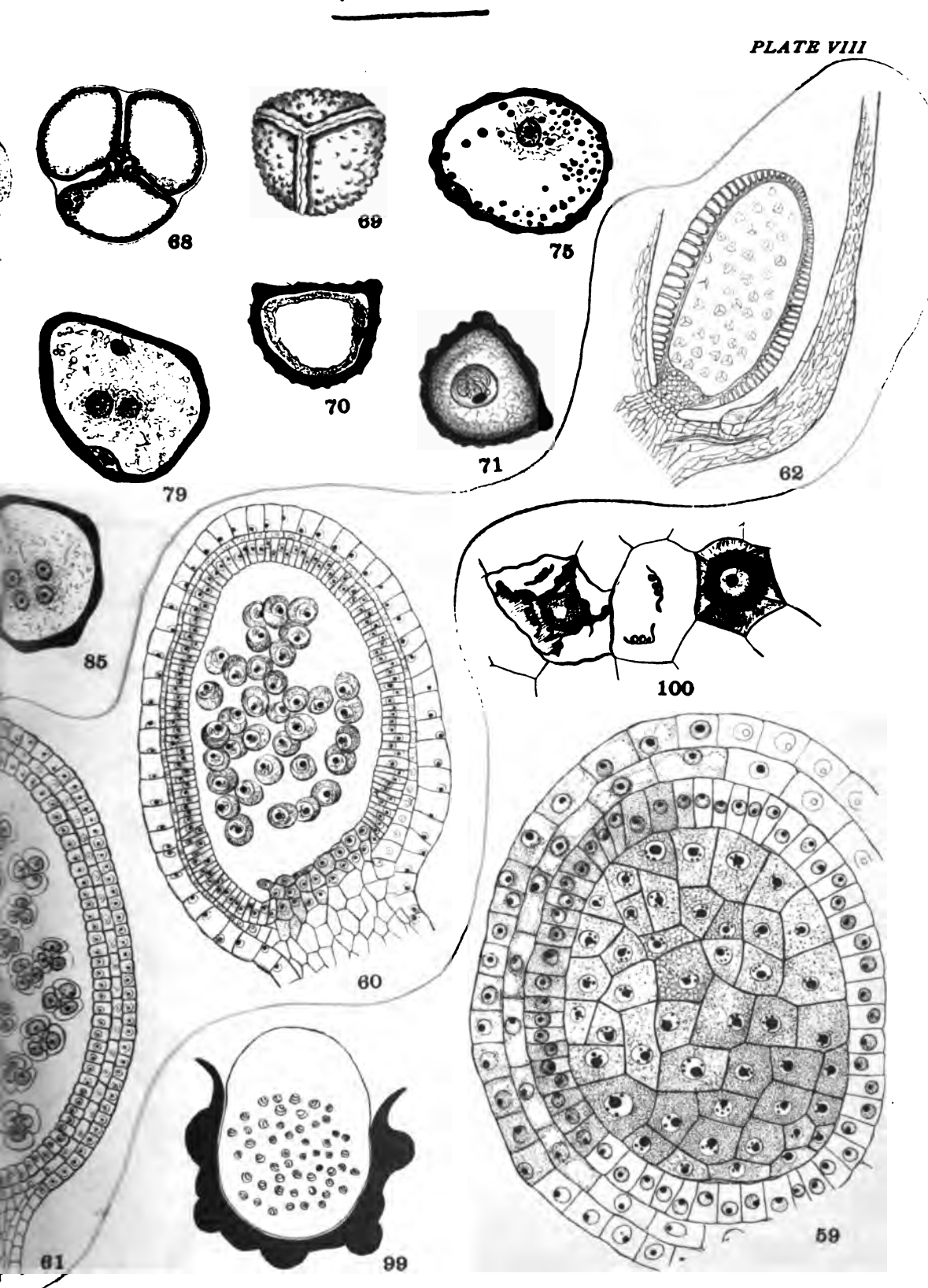
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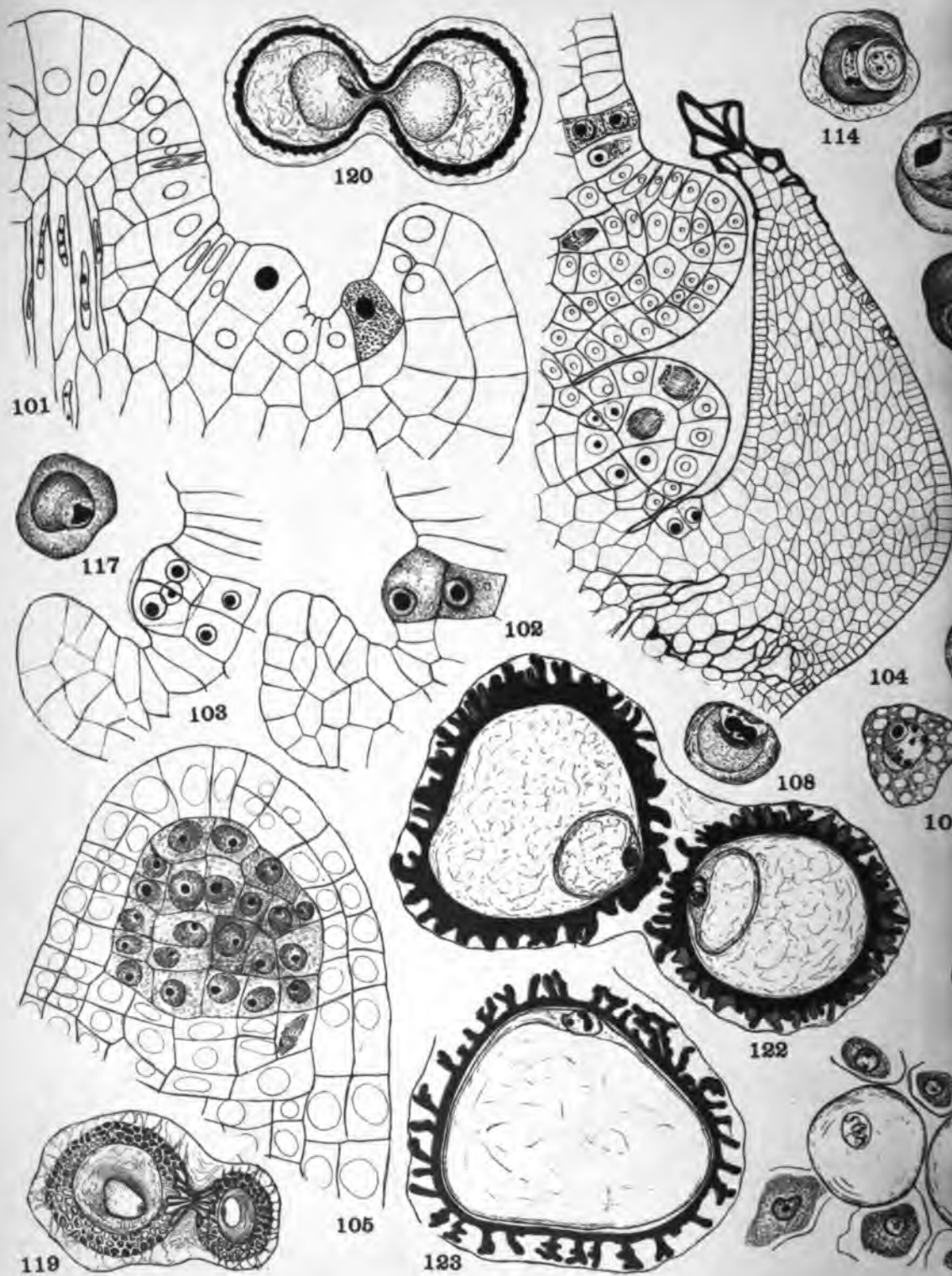




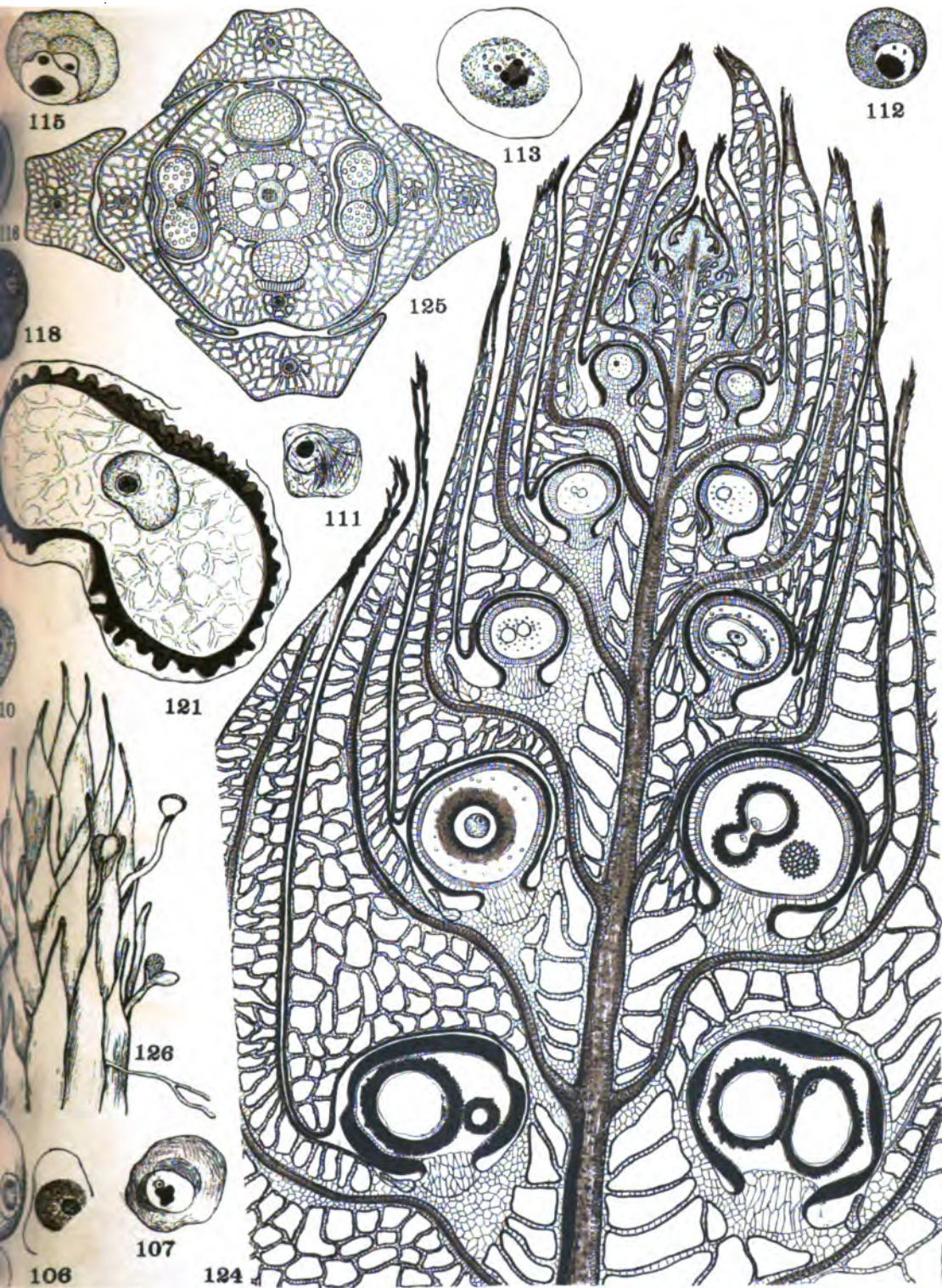
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eventually forms the initial sporangium wall. The hypodermal cell is the initial sporogenous cell. The first cell of the ligule is distinguished by a black nucleolus.

FIGS. 3 and 4. Radial section of portion of strobilus showing two superficial archesporial cells (shaded).

FIG. 5. Radial section of strobilus showing three-celled sporophyll (near apex), a two-celled sporangium (shaded) near the base of the subtending sporophyll, and the early stage of the vascular system.

FIG. 6. Radial section of strobilus showing four-celled sporangium possibly formed by two archesporial cells which divided by transverse walls. A stage subsequent to *figs. 3* and *4*.

FIG. 7. Radial section of older sporangium. Sporogenous cells are shaded, and are represented with black nucleoli.

FIG. 8. Vertical section of sporangium showing definite radial arrangement of cells and plane of cleavage dividing it into two groups of cells, each the progeny of one of the two superficial archesporial cells shown in *figs. 3* and *4*.

FIG. 9. Vertical median section of sporangium more advanced. Sporangium wall at this stage consists of one layer.

FIG. 10. Median transverse section of sporangium showing the sporangium cells, the tapetum, the inner and the outer layers of the sporangium wall.

FIG. 11. Section of sporangium after the spore mother cells are established. In this stage the sporogenous cells are distinguished by two conspicuous masses of granular matter which lie against the nucleus.

FIG. 12. Group of sporogenous cells in synapsis stage. Probably microspore mother cells.

FIG. 13. Detail of small portion of megasporangium showing megaspore mother cell (distinguished by the lumps of matter on either side of the nucleus) lying near the glandular tapetal cells. Two sterile sporogenous cells are at the left and below the megaspore mother cell.

FIGS. 14-24. Stages in the development of the megaspores. *Fig. 16* shows the characteristic sextuple spindle stage previous to the separation into spores, as seen in *fig. 18*. The four nuclei pass from the bases to the apices of the newly formed spores, as shown in *figs. 18-22*.

FIGS. 25-28. Details of cell division in the final stages of development of the female prothallium. *Fig. 25* represents a portion of a section of the stage represented in *fig. 49* to show thickness of protoplasmic vesicle and the invasion of fibrillae blocking off the separate nuclei.

FIG. 29. Section of archegonium. The egg and ventral canal cell are lying in a plane perpendicular to the long axis of the archegonium. The egg is the larger cell with black nucleus.

FIG. 30. Section of archegonium in which the egg lies below the ventral canal cell, and the neck canal cell above it. Two tiers of neck cells are drawn.

FIG. 31. Stage younger than that shown above, before neck cells are cut off.

FIG. 32. Archegonium with egg and ventral canal cell laterally placed. No trace of neck canal cell. Neck consists of four tiers of cells, each tier comprising an upper (superficial) cover cell, and one neck cell. The cells abutting on the venter belong to the prothallium.

FIGS. 33 and 34. Mature archegonia. In *fig. 33* a spermatozoid is lying on the receptive spot of the egg. *Fig. 34* shows a one-celled embryo.

PLATE VI. *Selaginella apus*. Oc. 2, oil immersion $\frac{1}{4}$.

FIG. 35. Section of young megasporangium at earliest stage when megaspore mother cell is distinguishable from sterile cells. For detail see *fig. 13*.

FIG. 36. Section of older stage of megasporangium. Megaspore mother cell has moved near center of sporangium.

FIG. 37. Oblique section of megasporangium showing young tetrad, and sterile mother cells, floating in slime composed in part of disintegrated sterile cells and in part of a secretion poured out by tapetum.

FIG. 38. Section of somewhat older stage of megasporangium containing group of four megaspores (only three are represented). Each spore consists of an outer coat, the exospore; a median layer which is later differentiated into the mesospore and the endospore; a vesicle of protoplasm containing a limpid fluid and possessing a very small nucleus. The spaces intervening between vesicle and median layer and between the latter and the exospore are filled with fluid.

FIGS. 39-41. Successive serial sections of a tetrad, to illustrate the fact that the exospore is a continuous envelope common to the group of spores and splits as they move apart.

FIG. 42. Section of group of megaspores but little older than those in *fig. 38*. Filamentous processes shown between spores. The mother cell membrane, beyond whose boundary the radiations do not pass, envelops the tetrad.

FIG. 43. Section of two megaspores more advanced. The spore at the left is cut nearly in half from apex to base, that at the right is a slice across the apex. Protoplasmic vesicle grown but little larger than in *fig. 42*. Spine-like processes upon exospore in connection with radiations. Mother cell membrane still evident.

FIG. 44. Section of the female gametophyte showing the protoplasmic vesicle with two nuclei. The radiations extend across the spaces between vesicle and median layer, between median layer and exospore, and between the latter and the original spore mother cell membrane.

FIG. 45. Section of female gametophyte whose vesicle has five nuclei and no cell walls. The exospore has grown to the size of the megasporangium as shown in *fig. 38*. The median layer has divided into the endospore (represented in section as a broad black band), and the mesospore which can be distinguished only as a delicate layer (represented by a single line) just without the endospore. The exospore is of spongy appearance and is still in contact with the persistent spore mother cell membrane. Fluid, presumably protoplasmic in nature, fills intervening spaces.

FIG. 46. A transverse median section of a female gametophyte in "film" stage. The megaspore, endospore and protoplasmic vesicle (studded in its apical region with large free nuclei) have stretched to the dimensions of the exospore, against whose inner surface they form a layer consisting of three delicate lamellae. The central vacuole is filled with clear fluid. The same stage is shown in *figs. 47* and *48* the former representing a surface view, the latter a median section.

PLATE VII. *Selaginella apus*.

All figures except 56 and 58 drawn with oc. 2, obj. $\frac{1}{4}$. *Fig. 56* is drawn with same combination as plate V; *fig. 58* with oc. 2, obj. $\frac{3}{4}$.

FIG. 47. Surface view of the protoplasmic vesicle as if seen through spore walls.

FIG. 48. Vertical median section of the same stage. The nuclei are massed in the apical region and the interior of the vesicle is occupied by a vacuole.

FIG. 49. Surface view of the gametophyte at the moment when protoplasmic fibrillae appear in the apical region and radiate over the surface of the gametophyte. The nuclei in the lower part of the figure are in process of division. The vesicle has increased in thickness (cf. *fig. 50*), and is beset with proteid granules. Contents of vacuole a homogeneous liquid.

FIG. 50. Median section of same.

FIG. 51. Median vertical section of female gametophyte. Protoplasmic vesicle much thicker in apical region where the nuclei are disposed in several layers diminishing to a single one in the equatorial region. Fibrillae permeating the nucleated portion have outlined indefinite areas but there are no cell walls. Contents of vacuole an emulsion.

FIG. 52. Section showing a portion of the megasporangium with female gametophyte *in situ* to show the differentiation of the wall. In the lowermost part of the figure the fragile cells of the area of dehiscence are seen in cross section. The inner layer of the wall probably supplies the outer with nourishment. The vestiges of the tapetum are upon its inner face, as a pavement layer, and a few sterile mother cells lie at the left next the wall.

FIG. 53. Vertical median section of female gametophyte showing the differentiation of cells into an apical superficial layer from which develop

archegonia, and a lower vegetative region. The delimitation of the archegonial region is shown by the trefoil shaped cleft seen in the lowermost spore of *fig. 58*.

FIGS. 54 and 55. Section of female gametophyte showing archegonia. Details of the process of division in the unshaded cells of *fig. 54* are represented in *figs. 26-28*.

FIG. 56. Two embryos, one of two cells, the other of three, lying side by side in one venter. All walls shown are parallel to the axis of the archegonium. Compare relative position of these embryos with that of the egg and ventral canal cell in *figs. 29, 32*.

FIG. 57. Embryo *in situ*. The root lies toward the apex of the gametophyte. At the right the club-shaped foot of large cells is embedded in the prothallial tissue. The embryo is bent with respect to its long axis so that the stem apex and cotyledons are brought nearer the root than appears in the drawing, which is a reconstruction from serial sections. The two leaves with their relatively large ligules are developed successively and envelop the tip of the stem (shown by dotted line). The embryo is still within the spore wall and sporangium.

FIG. 58. Vertical section of megasporangium containing three female gametophytes. The lowermost shows the trefoil-shaped cleft in the apical region of the spore wall, exposing the archegonia. The two other oblique sections are not so advanced in development. The pad of tissue at the base of the sporangium lies above a region of storage cells in contact with the vascular bundles of axis and sporophyte.

PLATE VIII. *Selaginella apus*.

All drawings made with oc. $\frac{3}{4}$, oil immersion, obj. $\frac{1}{12}$, excepting 60-62. Figs. 60, 61 were drawn with oc. 2, oil immersion, obj. $\frac{1}{12}$. Fig. 62 with oc. $\frac{3}{4}$, obj. $\frac{3}{4}$.

FIG. 59. Section of microsporangium, showing microspore mother cells, tapetum, inner and outer layers of sporangium wall.

FIG. 60. Median vertical section of microsporangium with mother cells in synapsis stage. Sub-archesporial pad developing at base of sporangium.

FIG. 61. Slightly oblique section of microsporangium showing microspores in groups of four (tetrads).

FIG. 62. Median vertical section of microsporangium to show sporangium wall at maturity. Dehiscence region cut across at apex. Sporangium opens in two valves, the subarchesporial pad at top of pedicel serving as a fulcrum.

FIGS. 63, 64. Microspore mother cells during first division of nucleus.

FIGS. 65, 66. Sextuple spindle stage with nuclear plates.

FIG. 67. Exterior view of young microspore.

FIG. 68. Section of tetrad, showing continuity of exospore around the four microspores, and spore mother cell membrane enveloping the tetrad. The protoplasm is a thin vesicle with a single nucleus, parietally placed, and surrounding a large central vacuole.

FIG. 69. Exterior view of mature microspore showing pebbled exospore.

FIG. 70. Vertical section of male gametophyte after first division of spore into generative and vegetative cells.

FIG. 71. Male gametophyte as seen by focusing through spore coats. The large generative nucleus lies near the wall, the vegetative cell not in focus.

FIG. 72. Male gametophyte showing generative cell in section.

FIG. 74. Male gametophyte showing lenticular vegetative cell at left. The remainder of the content constitutes the generative cell with a centrally placed nucleus. Masses of deeply staining proteid matter appearing.

FIG. 75. Section of male gametophyte in which masses of proteid matter have increased in number. Vegetative cell appressed to wall at left.

FIG. 76. The protoplasm is aggregated around the centrally placed generative nucleus and has sent out radiating processes, connecting it with a peripheral layer. The vacuoles thus isolated are filled with a semi-fluid granular matter.

FIGS. 77, 78. A common appearance, where a large symmetrical mass of proteid matter (solid black) in the central vacuole may be mistaken for a nucleus.

FIG. 79. First division of generative nucleus. Persistent vegetative cell appressed to wall in lower portion of figure.

FIGS. 80-87. Phases of the early divisions leading to the spermatozoid mother cells.

FIGS. 88-95. Sections in various planes showing the two cell complexes and their relation to the dissolving proteid masses by which they are enveloped. The masses of food matter are separated by strands of protoplasm in *figs. 88, 93, 95*.

FIG. 96. Exterior view of male gametophyte displaying trefoil shaped cleft in apical portion. The complexes of spermatozoid mother cells protrude through the gap.

FIG. 97. Male gametophyte with spermatozoid mother cells as seen by focusing through spore coats. The unusually persistent vegetative cell is represented lying above the mother cells.

FIGS. 98, 99. Male gametophyte at maturity. The endospore protrudes like a short pollen tube through a gap on the ruptured exospore, and contains a slimy homogeneous fluid which later is discharged with the spermatozooids.

FIG. 100. A transverse section of a female gametophyte displaying the

eggs and spermatozooids. The necks of the archegonia have been sliced off somewhat obliquely.

PLATE IX. *Selaginella rupestris*.

FIG. 101. Apex of a strobilus in radial section. A single archesporial cell (shaded) at the base of a sporophyll. The cell with black nucleus is the initial cell of the next younger sporophyll of the same rank.

FIG. 102. First division of archesporial cell into a hypodermal and a superficial cell.

FIG. 103. The superficial cell of the first division has divided into three wall cells, and the hypodermal cell into two sporogenous cells, forming a wedge-shaped mass.

FIG. 104. Radial section of portion of strobilus. The largest sporophyll has ceased to grow at the apex which is converted into a branched spine. The initial cells of the ligule at the base of the sporophyll are represented with black nuclei. The tissue is rupturing to form air chambers in the basal region. The sporangium has been outstripped in growth by the sporophyll next above it. The two shaded cells in the upper part of the figure represent the same stage of sporangium as seen in *fig. 102*.

FIG. 105. Section of sporangium. The wall is nearly completed and the tapetum is becoming differentiated from the sporogenous complex of cells. The limits of the latter in the basal region not so clearly defined as represented in the figure.

FIG. 106. Detail showing two megaspores and several sterile mother cells.

FIGS. 107-117. Phases undergone by megaspore mother cells preceding divisions into spores.

FIG. 118. First division of megaspore mother cells into two spores.

FIG. 119. Section through sister megaspores. A portion of the sculptured exospore is removed from the upper surface of each, showing the protoplasmic vesicle with a small nucleus floating in liquid. The spore mother cell membrane envelops the two spores.

FIG. 120. Section showing incomplete division of the protoplasm of two megaspores. The nucleus of the spore mother cell has not divided.

FIG. 121. Section through a single megaspore whose contour and large nucleus suggest that it is the direct product of a spore mother cell which has failed to divide.

FIGS. 122, 123. Older stages of megaspores.

FIG. 124. Median vertical section of tip of strobilus showing relation of sporangia to sporophylls and vascular system. The meristematic regions at the bases of the sporangium pedicels grow upward slightly by causing the megasporangia to appear sunken in the hollows thus formed. There were only megasporangia in this strobilus.

FIG. 125. Transverse section of strobilus through microsporangia. In the center of the figure is the axial strand surrounded by air chambers interspersed with meristematic tissue. At the right and left are two microsporangia, each showing the septum which incompletely separates it into two loculi. The rest of the diagram represents the cross sections of six sporophylls with their ligules.

FIG. 126. Apical portion of strobilus showing young sporophytes protruding from undetached megasporangia.

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